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Interrelationships Between Meloidogyne Incognita and Rotylenchulus Reniformis on Soybean.

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INTERRELATIONSHIPS BETWEEN *MELOIDOGYNE INCOGNITA* AND *ROTYLENCHULUS RENIFORMIS* ON SOYBEAN

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
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in partial fulfillment of the
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in

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ABSTRACT

The replacement series approach was used to evaluate competition between Meloidogyne incognita (Mi) and Rotylenchulus reniformis (Rr). In greenhouse tests, soil in pots containing 'Davis' soybean was not infested or infested with 1,000 vermiform nematodes in the following Mi:Rr ratios: 100:0, 75:25, 50:50, 25:75, and 0:100. After 86 days, relative nematode yields (RNYs) (number of each species in mixed culture divided by number in nonmixed culture) were calculated based on soil populations. Calculated values were plotted and the resulting line compared with a reference line representing equal inter- and intraspecific competition. RNYs for Mi were higher than predicted where Mi and Rr occurred together, suggesting increased reproduction in the presence of Rr. RNYs for Rr did not differ from predicted yields, indicating no effect of Mi on Rr. These relationships were not detected using analysis of variance and were independent of host colonization by Diaporthe phaseolorum var. caulivora.

To determine if larger Mi soil populations resulted from increased reproduction or from migration, a grinding technique was developed to liberate vermiform and swollen nematodes from roots. Experiments on soybean and tomato evaluated the efficiency of method (stir, grind), carrier (water, 0.5% NaOCl), and duration (1X, 2X) on extraction of nematodes and eggs. Grinding liberated more nematodes than stirring, but the reverse was true for egg recovery. Among grinding treatments, a duration of 10 sec in 0.5% NaOCl provided the most efficient nematode recovery.

The effect of soybean genotype on competition between Mi and Rr was evaluated in greenhouse and microplot replacement series experiments on 'Davis' (susceptible to Mi) or 'Buckshot 66' (resistant to Mi) as described previously. After 91 days, the RNY of each species was calculated based on combined soil and root

populations. On 'Davis', Mi greenhouse populations were larger in the presence of Rr. In microplots, small Mi and Rr populations likely resulted from severe galling and destruction of feeder roots. On 'Buckshot 66', Rr did not affect Mi greenhouse and microplot populations. With the single exception noted for 'Davis' in the microplot, Rr populations were not influenced by competition with Mi.

CHAPTER 1

INTRODUCTION

The United States is responsible for half of the total world production of soybean (Glycine max (L.) Merrill) (Morrison and McCormick, 1996). In the southern U.S., 7.28 million hectares of soybean were harvested annually from 1988 to 1993. During this period, annual yield losses due to diseases ranged from 9.8-15.2% and cost growers 9.52 million metric tons of soybeans, or almost $2.3 billion (Sciumbato, 1993; Wrather and Sciumbato, 1995). In Louisiana, where 404,858 to 417,004 hectares were planted annually between 1992 and 1995, soybean ranks fifth among plant commodities in total crop value (Anonymous, 1993; Anonymous, 1994; Anonymous, 1995; Anonymous, 1996). Diseases caused by nematodes and fungi are responsible for a large proportion of the annual losses in soybean. Nematodes caused estimated annual losses of 4-8% of the Louisiana soybean crop between 1988 and 1993, while fungi are estimated to reduce Louisiana soybean yields 6-12% annually (Sciumbato, 1993; Wrather and Sciumbato, 1995).

More than 100 phytoparasitic nematode species have been reported in association with soybean roots (Sinclair and Backman, 1989). Foliar symptoms of nematode damage frequently mimic those of water and nutrient deficiencies, i.e., wilting, stunting, and chlorosis, and usually occur in patches within a field (Agrios, 1988; Sinclair and Backman, 1989). Soybean root systems parasitized by nematodes generally have fewer feeder roots, necrotic lesions or discolored areas, and may show reduced nodulation (Sinclair and Backman, 1989). In addition to these symptoms, colonization of the roots by nematodes in the genus Meloidogyne results in the formation of distinctive knots or galls (Overstreet, 1996; Sinclair and Backman, 1989).

Root-knot nematode (Meloidogyne incognita (Kofoid & White) Chitwood race 2) and reniform nematode (Rotylenchulus reniformis Linford & Oliviera) are pathogenic to soybean (Sinclair and Backman, 1989). Cotton (Gossypium hirsutum L.), another

major Louisiana crop susceptible to damage by these nematodes (Overstreet and McGawley, 1995, 1996), often is grown in rotation with soybean. The shared host range and geographic distribution of root-knot and reniform nematodes increase the likelihood that the two species compete for host root tissue.

Meloidogyne incognita attacks more than 2,000 cultivated plant species worldwide, and is the predominant root-knot species in Louisiana (Overstreet, 1996). The life cycle of root-knot nematode includes an egg stage, four juvenile stages, and an adult stage and requires 3 to 4 weeks for completion. The second stage juvenile is infective. Females are swollen, sedentary endoparasites, producing up to 500 eggs in a gelatinous matrix. Reproduction is through amphimixis.

Rotylenchulus reniformis has a more limited host range than M. incognita, attacking 140 species of plants (Overstreet, 1996). The life cycle of reniform nematode is similar to that of M. incognita. The preadult female (fourth stage juvenile) is the only infective stage. Females are swollen, sedentary semiendoparasites, producing up to 40 eggs in a gelatinous matrix. Reproduction is through amphimixis.

More than 124 species of fungi have been reported in association with soybean, including at least 62 pathogens (Sinclair and Backman, 1989). In Louisiana, 15 fungal diseases occur commonly (Whitam, 1996). Symptoms of fungal diseases are quite varied and include spots, blights, rots, mildews, cankers, wilts, anthracnose, and damping-off. Signs of phytopathogenic fungi, such as sclerotia, mycelia, and sori, may also be evident.

The fungus Diaporthe phaseolorum (Cke. & Ell.) Sacc. var. caulivora Athow & Caldwell causes stem canker of soybean. Louisiana soybean losses attributed to this pathogen ranged from 0.5 to 4.0% between 1988 and 1993 (Sciumbato, 1993; Wrather and Sciumbato, 1995), though complete crop losses are possible if a susceptible cultivar

is attacked (Sinclair and Backman, 1989; Whitam, 1996). The disease is initiated early in the season from ascospores produced in perithecia on plant debris. All soybean varieties may be infected, but only susceptible ones will show symptom development. The disease is characterized by the development of reddish-brown lesions on stems that expand and darken as the season progresses (Backman et al., 1985). After the plants enter the reproductive stages of development, interveinal chlorosis and necrosis appear (Ploetz and Shokes, 1985; Sinclair and Backman, 1989). Toxins have been implicated in development of foliar symptoms (Burra, 1988; Lalitha et al., 1989; Sinclair and Backman, 1989). Plant death is attributed to the girdling effect of the canker (Backman et al., 1985; Whitam, 1996).

The soybean crop is under simultaneous attack by a variety of pests, including weeds, insects, and pathogens (Adeniji et al., 1975; Alston et al., 1993; Appel et al., 1984; Barker et al., 1972; Browde et al., 1994; Bustillo, 1972; Guy and Lewis, 1987; Herman et al., 1988; Ibrahim and Lewis, 1986; Lawn and Noel, 1986; McLean and Lawrence, 1993; Meredith et al., 1983; Niblack et al., 1986a,b; Overstreet et al., 1990; Pacumbaba, 1992; Padgett et al., 1994; Robbins et al., 1990; Ross, 1965; Roy et al., 1989; Russin et al., 1986, 1989a,b, 1990, 1993; Singh, 1976). The first report of a pathogen complex was published more than 100 years ago, when Atkinson (1892) described an increase in the severity of Fusarium wilt on cotton plants simultaneously colonized by root-knot nematodes. Since that time, the literature has included many reports of pathogen complexes involving nematodes which have been summarized by Jenkins and Taylor (1967), Khan (1993), McGawley (1991), and Powell (1971).

Interactions between pathogens may be defined ecologically, i.e., in terms of their effects on each other, or etiologically, i.e., in terms of their effect on producing disease in their host. The two are often related. Disease severity often is a function of

population size. One juvenile nematode, one bacterial cell, one fungal spore, or one virus particle does not pose an immediate threat to the host. However, the activities of the pathogen as it infects, grows, spreads, and ultimately reproduces within the host tissue often result in disease development.

When organisms share a similar ecological niche, as is often the case with phytoparasitic nematodes, they may compete directly for space and nutrients. This type of interaction generally results in the suppression of one or both species (Eisenback, 1993). A second possibility is that the relationship between pathogens living in the same microenvironment may be beneficial to one or both. There are numerous reports of nematodes predisposing plants to infection by wilt-inducing fungi and root-rot fungi (Powell, 1971). Mechanisms behind the increased suitability of the host include the physical wound as a port of entry and histological changes such as the formation of syncytia that provide an environment conducive to colonization by other organisms (Powell, 1971). Split-root experiments suggest that pathogens colonizing different parts of the same plant may also impact each other through a modification of the host's physiology (Eisenback, 1993). The ecological association between species impacts their survival and reproduction, thereby influencing the etiological manifestation of the relationship between them.

Ecological and etiological relationships between phytoparasitic nematodes are defined within the context of the host. Pre-infection host resistance mechanisms include attraction or repulsion of nematodes or hatch of nematode eggs in response to root exudates (Huang, 1985; Veech, 1981), presence of nematicidal compounds in host tissues (Huang, 1985; Veech, 1981), and the morphology and anatomy of the host root system including abundance of lateral roots and lignification of root tissue (Fassuliotis, 1982). Pre-infection resistance mechanisms are important in determining which

nematode species are likely to coinhabit a given host. Post-infection resistance mechanisms include preformed chemicals such as phenolics (Huang, 1985; Veech, 1981), synthesis of phytoalexins and their accumulation in challenged tissues (Huang, 1985; Veech, 1981), initiation of a hypersensitive reaction (Huang, 1985; Veech, 1981), and alterations in the nutritional status of the host (Fassuliotis, 1982; Huang, 1985; Veech, 1981). It is possible that these mechanisms may be modified after penetration by one nematode species to inhibit or favor development of a second nematode species.

Competition between nematodes has typically been evaluated by comparing the number of individuals recovered from the soil using analysis of variance (ANOVA) and appropriate post-ANOVA means separation procedures (Chapman and Turner, 1975; Dickson and McSorley, 1990; Gay and Bird, 1973; Guy and Lewis, 1987; Herman et al., 1988; Ibrahim and Lewis, 1986; Niblack et al., 1986b; Thomas and Clark, 1983a,b; Wallace, 1983). However, a means comparison test may not account for differences in the inherent reproductive capabilities of individual species. Recently, replacement series techniques developed by plant ecologists (de Wit, 1960; de Wit et al., 1966) have been employed to study interactions between phytopathogenic fungi (Adee et al., 1990; Zitko and Timmer, 1994), epiphytic ice-nucleating bacteria (Wilson and Lindow, 1994b), bacteria existing in the bean phyllosphere (Wilson and Lindow, 1994a), and antagonists to Penicillium expansum coexisting in wounds on apple fruit (Janisiewicz, 1996). Replacement series experiments are designed to quantitatively assess the relative impact of inter- and intraspecific competition between two species introduced alone or together in various ratios at a single community density. Rather than employing actual population counts, calculated relative yields (number of individuals of a species in mixed culture expressed as a proportion of the same species in nonmixed culture) are

plotted against the input proportion of each species. If inter- and intraspecific competition are equal, final nematode population sizes for each species will be directly proportional to the percentage of that species initially introduced.

Because the geographic distribution of root-knot and reniform nematode overlap in Louisiana, investigations concerning the combined effects of these species on each other and on their soybean host were conducted. The primary objective of this research was to examine the ecological relationship between root-knot and reniform nematode on soybean and describe related etiological effects. Secondary objectives were to evaluate the applicability of the replacement series technique in nematode competition studies and to examine the influence of the stem canker fungus and soybean genotype on the relationship between root-knot and reniform nematode.

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CHAPTER 2

REPLACEMENT SERIES: A TOOL FOR CHARACTERIZING COMPETITION BETWEEN PHYTOPARASITIC NEMATODES

Introduction

Root-knot (Meloidogyne incognita (Kofoid & White) Chitwood race 2) and reniform (Rotylenchulus reniformis Linford & Oliviera) nematodes are pathogenic to soybean (Glycine max (L.) Merrill) (Sinclair and Backman, 1989). In Louisiana, nematode damage reduced soybean yields 4-8% annually during 1988-1993 (Sciumbato, 1993; Wrather and Sciumbato, 1995). Cotton (Gossypium hirsutum L.), another major crop susceptible to damage by these nematodes (Overstreet and McGawley, 1995, 1996), is grown in many of the same areas in Louisiana as soybean. Since the host range and geographic distribution of root-knot and reniform nematodes overlap, it is likely that the two species compete for host root tissue.

Competition between nematodes typically has been evaluated by comparing the number of individuals recovered from the soil using analysis of variance (ANOVA) and appropriate post-ANOVA means separation procedures (Chapman and Turner, 1975; Dickson and McSorley, 1990; Gay and Bird, 1973; Guy and Lewis, 1987; Herman et al., 1988; Ibrahim and Lewis, 1986; Niblack et al., 1986; Thomas and Clark, 1983a,b). However, a means comparison test may not account for differences in the inherent reproductive capabilities of individual species.

Recently, replacement series techniques developed by plant ecologists (de Wit, 1960; de Wit et al., 1966) have been employed to study interactions between phytopathogenic fungi (Adee et al., 1990; Zitko and Timmer, 1994), epiphytic ice-nucleating bacteria (Wilson and Lindow, 1994b), and bacteria existing in the bean phyllosphere (Wilson and Lindow, 1994a). Replacement series experiments are designed to quantitatively assess the relative impact of inter- and intraspecific competition between two species at a single community density. Species are introduced alone or together in various ratios. At the end of the experiment, relative nematode

yields (number of each species in mixed culture divided by number in nonmixed culture) are calculated for each species. Inhibition or stimulation of a species can be visualized by plotting the relative nematode yields against the input proportion of that species (Figure 2.1). If inter- and intraspecific competition are equal, final nematode population sizes for each species should be directly proportional to the percentage of that species initially introduced.

The primary objective of this research was to assess the usefulness of the replacement series technique in detecting and describing competitive interactions between root-knot and reniform nematode. A secondary objective was to determine if the relationship between root-knot and reniform nematode differs on soybeans colonized by the stem canker fungus, Diaporthe phaseolorum (Cke. & Ell.) Sacc. var. caulivora Athow & Caldwell (Dpc). A preliminary report has been published (Erwin et al., 1995).

Materials and Methods

General procedures: Experiments were conducted in a greenhouse, where temperatures ranged from 22-35°C. Supplemental incandescent and fluorescent lighting (ca. 260 $\mu E \cdot m^{-2} \cdot sec^{-1}$) provided a minimum of 14 hours of light daily. These studies utilized 15-cm-diam. clay pots that contained approximately 1.6 kg of a soil mixture composed of three parts fumigated (67% methyl bromide, 33% chloropicrin) Convent silt loam soil (Aeric Fluvaquent, coarse-silty, mixed, nonacid, thermic) and two parts autoclaved sand.

Soybean cv. Davis seeds were treated with a commercial preparation of Bradyrhizobium japonicum (Kirchner) Jordan (Nitragin; The Nitragin Co., Milwaukee, WI) and sown in flats. Seedlings of uniform size were selected when plants were at growth stage V1 (Fehr et al., 1971), and a single seedling was transplanted to each pot.

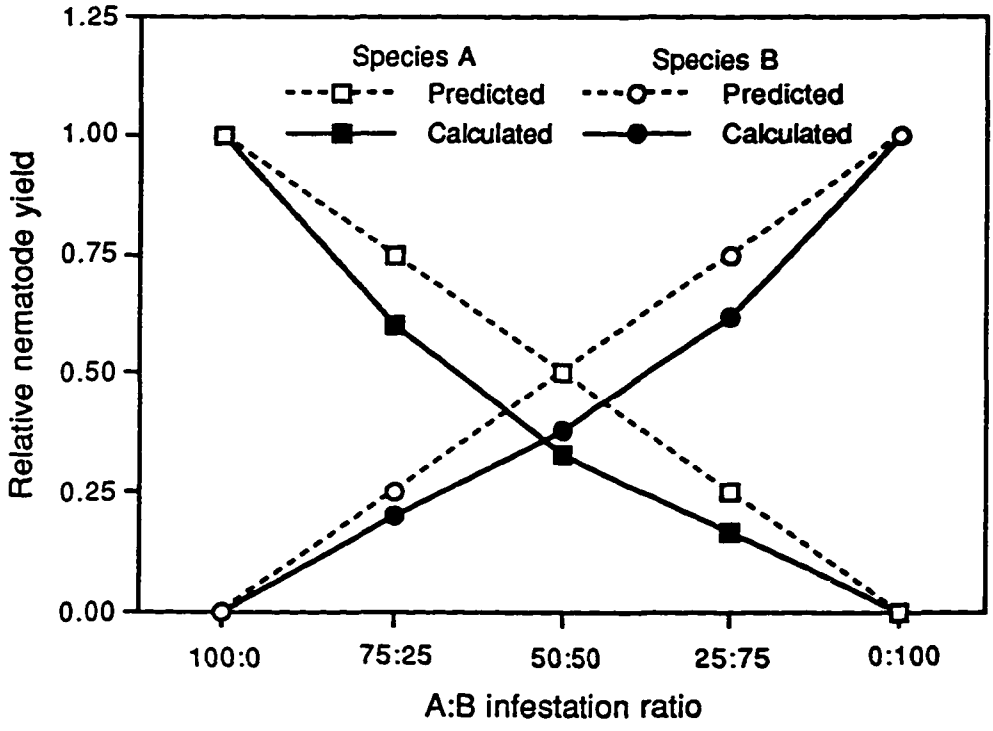

Fig. 2.1. Hypothetical replacement series between species A and B. Equivalence of inter- and intraspecific competition is indicated by dotted lines. Interspecific competition (inhibition) is illustrated by solid lines.

Plants were fertilized every 14-21 days with 120 ml of a 23-19-17 N-P-K fertilizer solution (RapidGro; Chevron Chemical Co., San Ramon, CA), beginning at transplant. Plants received approximately 26 ppm N, 20 ppm P, and 33 ppm K at every fertilization.

Root-knot and reniform nematode populations were derived from single egg masses and maintained on tomato (Lycopersicon esculentum L. 'Rutgers') in a greenhouse. Inoculum consisted of vermiform nematodes obtained from soil by hand-sieving and centrifugal flotation (Jenkins, 1964). Soil in each pot was infested with the required number of each species by pipetting nematodes suspended in tap water into two depressions made in the soil. Tap water was pipetted into depressions in control pots. Each depression was 1 cm in diameter, 4 cm deep, and 5 cm from the base of the stem on opposite sides of the plant. After infestation, the depressions were filled with additional soil mixture.

At the end of each experiment, five soil cores (2.5-cm-diam.) from the soil surface to the bottom of the pot were collected from each pot, mixed thoroughly, and subsampled (150 g) for nematode extraction. Nematodes were extracted by hand-sieving and centrifugal flotation. Numbers of juveniles, males, vermiform females, and swollen females collected on a 38-μm-pore sieve were recorded for each species.

Plant stems were cut at the soil surface and the root-soil mass was removed from each pot. Root systems were freed from soil by gently washing in tap water. Root-knot gall severity was rated according to the following scale: 0 = no galls, 1 = galls less than 3 mm in diameter with no reduction in the number of feeder roots, 2 = galls 3-10 mm in diameter with no reduction in the number of feeder roots, 3 = galls 10-20 mm in diameter with no or slight reduction in the number of feeder roots, 4 = galls >20 mm in diameter with moderate reduction in the number of feeder roots, and 5 = galls >20 mm

in diameter with major reduction in the number of feeder roots. Root-knot gall incidence was rated according to the following scale: 0 = no galls, 1 = galls confined to 25% or less of the root system, 2 = galls appearing over 26-50% of the root system, 3 = galls appearing over 51-75% of the root system, and 4 = galls appearing on 76% or more of the root system.

Nematode eggs were extracted from a subsample (2 g) removed at random from each root system following a procedure modified from Hussey and Barker (1973). Root tissue was stirred continuously for 10 min in 0.5% NaOCl then poured onto nested 75- and 25-μm-pore sieves. Eggs collected on the 25-μm-pore sieve could not be identified to species, so egg counts were not included in population totals.

Cultures of Dpc were maintained on 2% water agar (WA) (Difco, Detroit, MI) or potato dextrose agar (PDA) (Difco, Detroit, MI) at room temperature (22-26°C) in the laboratory. Fungus cultures were grown on PDA for 7-10 days before transferring to inoculum preparation plates. The toothpick inoculation procedure described by Russin et al. (1986) was employed. Inoculated plants received toothpick sections infested with Dpc, while sterile toothpick sections were inserted into control plants. The Dpc isolate used was collected in 1989 at the Burden Research Plantation, Baton Rouge, LA, from soybean cv. Bedford (G. B. Padgett, pers. comm.). Virulence of this isolate was maintained through annual inoculation of and reisolation from a susceptible soybean variety.

Stem canker lesion length (mm) was recorded at 10-day intervals for 40 days, beginning 10 days after inoculation. Tissue sections collected from the margins of the lesion (symptomatic plants) or near the inoculation site (asymptomatic plants) were surface-disinfested by soaking for 5 min in 0.5% NaOCl. Sections were rinsed in sterile distilled water, blotted on sterile paper towels, and plated on WA. Fungi

growing from these sections were transferred to PDA and identified as Dpc based on colony morphology, development of pycnidia or perithecia, and morphology of conidia or ascospores.

Replacement series experiments: Nematode and fungus treatments in a factorial arrangement were examined using a randomized complete block design with five replications. Root-knot and reniform nematodes were introduced alone or in combination at an initial community density of 1,000 individuals per pot when plants reached growth stages V2 - V3. Soil was not infested or nematodes were introduced at one of the following root-knot:reniform ratios: 100:0, 75:25, 50:50, 25:75, and 0:100. Forty-one days after infestation (R1 or R2 in tests 1 and 2), plants were inoculated with Dpc. Experiments were terminated 84-86 days after nematode infestation (R5 or R6 in tests 1 and 2, R2 in test 3). At harvest, plants were divided into root and shoot portions by cutting the stem at the soil line. Soybean root and shoot dry weights (after drying at 70°C for 4 days) were recorded after galling assessment and collection of tissue samples for egg extraction and fungus reisolation. Soil samples were processed and nematodes counted. Relative nematode yields were calculated based on the number of nematodes of each species extracted from soil, expressed per gram of dry root tissue. For these experiments, relative yield was calculated by dividing the number of nematodes of a species recovered from mixed culture by the number of nematodes of the same species recovered from nonmixed culture. This experiment was conducted twice (tests 1 and 2). After determining that the fungus did not influence the relationship between the nematodes, the experiment was conducted a third time with the Dpc treatments omitted (test 3). Ten replications were used in each experiment.

Confirmation of proposed relative nematode yields: A preliminary experiment was conducted once to evaluate the accuracy of predicted relative nematode yield lines as

dictated by the replacement series model. The model assumed that each species would yield in direct proportion to its initial inoculum level, so a stepwise, linear relationship in relative nematode yield is predicted. To document the inherent reproductive capacity of each species, root-knot and reniform nematodes were grown in monospecific culture at an infestation density of 1,000/pot (full density) or at reduced densities of 750, 500, or 250/pot. Each treatment was replicated 10 times in a randomized complete block design. Relative nematode yields were calculated based on the number of nematodes of each species extracted from soil. For this experiment, relative nematode yield was calculated for each species at each infestation density by dividing the number of nematodes recovered in reduced-density cultures by the number of nematodes recovered in the full-density culture.

Data presentation and analyses: To evaluate replacement series for assessing competition between root-knot and reniform nematodes, results obtained with this technique were compared with those from the traditional ANOVA. Analysis of variance and Tukey's HSD means separation procedures were performed on nematode numbers and relative nematode yields ("Fit Model" and "Fit Y by X" modules of SAS JMP version 3.0) (SAS Institute, 1994). Differences between the predicted relative yield lines defined by the replacement series model and the relative nematode yield lines plotted using calculated relative nematode yield values were determined using lack-of-fit regression ("Fit Model" module of SAS JMP version 3.0) (SAS Institute, 1994). Paired t-tests ("Fit Y by X" module of SAS JMP version 3.0) (SAS Institute, 1994) were used to determine at which ratio(s) the predicted and calculated relative nematode yield values differed. Plant weights, galling indices, and stem canker lesion length data were analyzed using ANOVA and Tukey's HSD means separation procedures so that values could be compared with those of the noninoculated control.

Results

In monospecific culture, root-knot and reniform relative nematode yields were linear and proportional to initial inoculum levels. Lines based on calculated relative nematode yield values did not differ ($P \leq 0.05$) from those predicted by the replacement series model for either root-knot or reniform nematode. The relative nematode yield lines predicted in the replacement series model accurately represented the inherent reproductive capability of the nematodes. Therefore, the predicted relative nematode yield values defined by the model were used as the reference values in replacement series experiments involving combinations of these species.

In mixed species experiments, lack-of-fit regression indicated that the relative nematode yield line for root-knot nematode did not fit the reference line predicted by the replacement series model ($F = 5.9401$, $P = 0.0008$) (Figure 2.2). Paired t-tests established that the calculated relative nematode yields were significantly ($P \leq 0.05$) higher than the predicted values at all ratios where root-knot and reniform nematode occurred together. The apparent enhancement of root-knot nematode reproduction did not correspond to a decrease in the reniform population; the calculated relative nematode yield line for this species did not differ from the reference line ($F = 0.7565$, $P = 0.5203$) (Figure 2.2).

In tests 1 and 2, inoculation with Dpc resulted in measurable canker development 20, 30, and 40 days after inoculation. Canker lengths 40 days after inoculation, which represent the cumulative effect of the fungus, averaged 139.4 mm and were not influenced by colonization of the host by either or both nematodes ($F = 1.3726$, $P = 0.2763$). Root, shoot, and total dry weights for plants inoculated with the fungus were 7.8, 3.8, and 11.6 g lower, respectively, than noninoculated controls (Table 2.1). Inoculation with Dpc reduced galling severity and incidence (Table 2.1).

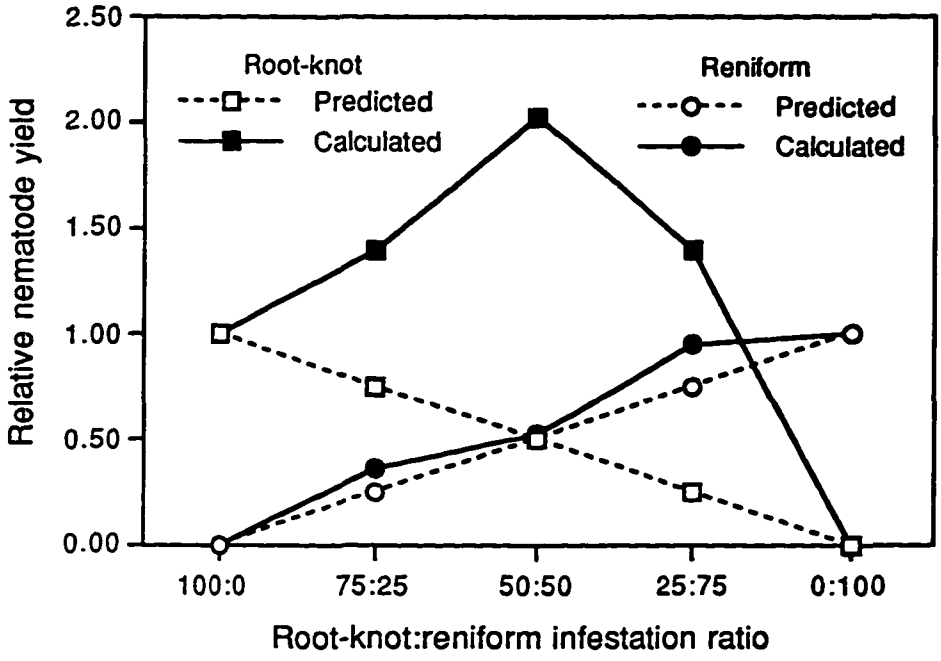

Fig. 2.2. Relative nematode yield of root-knot and reniform nematodes from soil 84-86 days after infestation on 'Davis' soybean in greenhouse tests. Calculated data points are means of 30 replications in three tests. Predicted reference values indicate the expected response if inter- and intraspecific competition are equal.

Table 2.1. Influence of test, inoculation with Diaporthe phaseolorum var. caulivora (Dpc), root-knot:reniform infestation ratio, and their interactions on soybean cv. Davis weight, nematode soil populations, relative yield values, and root galling induced by Meloidogyne incognita race 2 84-86 days after nematode infestation in two greenhouse experiments.

		Dry weight (g)			Nematodes/g dry root		Relative yield		Root gall rating	
Treatment	Level	Root	Shoot	Plant	Root-knot	Reniform	Root-knot	Reniform	Severity[a]	Incidence[b]
Test	1	31.9	11.9	43.8	584	768	1.41	0.55	3.3	3.7
	2	17.8	21.5	39.3	2,109	4,461	1.57	0.88	2.7	3.2
Dpc	Yes	20.9	14.8	35.7	1,442	2,268	1.04	0.71	2.6	3.1
	No	28.7	18.6	47.3	1,252	2,961	1.94	0.72	3.3	3.8
Mi:Rr	0:0	23.4	24.8 a	48.2	—	—	—	—	—	—
Ratio	100:0	23.4	12.8 bc	36.2	1,512	—	1.00	—	3.2	3.6
	75:25	21.7	11.7 c	33.4	1,862	2,653 b	1.74	0.37 b	2.8	3.4
	50:50	34.9	15.9 bc	50.8	979	2,078 ab	2.05	0.48 a	3.0	3.4
	25:75	23.4	15.3 bc	38.7	1,034	2,653 ab	1.18	1.00 a	3.1	3.4
	0:100	22.3	19.6 ab	41.9	—	4,619 a	—	1.00 a	—	—
Source:										
Test		***	***	NS	***	***	NS	NS	***	*
Dpc		**	***	***	NS	NS	*	NS	***	***
Mi:Rr Ratio		NS	***	*	NS	**	NS	*	NS	NS
Test × Dpc		NS	*	NS	NS	NS	NS	NS	**	***
Test × Mi:Rr ratio		NS	NS	NS	NS	NS	NS	NS	**	NS
Dpc × Mi:Rr ratio		NS	NS	NS	NS	NS	NS	NS	NS	NS

Means followed by the same letter do not differ (Tukey's HSD, $P \leq 0.05$).

[a] Severity is rated on a 0-5 scale where 0 = no galls and 5 = galls > 20 mm in diameter with major reduction in the number of feeder roots.

[b] Incidence is rated on a 0-4 scale where 0 = no galls and 5 = galls appearing on ≥ 76% of the root system.

*, **, and *** indicate significant differences at $P \leq 0.05$, 0.01, and 0.001, respectively. NS = means are not significantly different.

An average of 500 fewer eggs were recovered per gram of root tissue from plants colonized by Dpc than from respective controls. In spite of stem canker symptom development and successful reisolation of the fungus from inoculated plants, Dpc did not affect root-knot or reniform soil population densities and no infestation ratio by fungus interactions were detected with regard to soil populations (Table 2.1). Relative nematode yields for MI were lower for populations developing on plants with stem canker, but this effect was consistent across all nematode ratios (Table 2.1). Consequently, Dpc and related interaction terms were removed from the model and their associated variances pooled with that of the model error term. This allowed data from tests 1 and 2 to be pooled with data from test 3 for subsequent ANOVA and regression analyses.

Soybean root, shoot, and plant weights varied significantly among the three tests (Table 2.2). Nematode infestation ratio did not impact root or plant weights, but contributed to a significant test by ratio interaction with respect to shoot weight (Table 2.2). In two of the three tests, shoot weights were significantly lower than the 0:0 control on plants inoculated with high levels (100:0, 75:25) of root-knot nematode (Figure 2.3).

Nematode population densities and root gall indices varied significantly between tests (Table 2.2). In general, reniform nematodes outnumbered root-knot nematodes in all mixed-species treatments. More reniform nematodes were extracted from pots inoculated only with this species than from pots containing mixtures of reniform and root-knot nematode (Table 2.2). The test by ratio interaction (Table 2.2, Figure 2.4) indicated that severity of galling on plants colonized by both nematode species was generally equivalent to that seen on plants colonized only by root-knot nematode. The

Table 2.2. Influence of test, root-knot:reniform infestation ratio, and their interactions on soybean cv. Davis weight, nematode soil populations, relative yield values, and root galling induced by Meloidogyne incognita race 2 84-86 days after nematode infestation in three greenhouse experiments.

		Dry weight (g)			Nematodes/g dry root		Relative yield		Root gall rating	
Treatment	Level	Root	Shoot	Plant	Root-knot	Reniform	Root-knot	Reniform	Severity[a]	Incidence[b]
Test	1	31.9 a	11.9 a	43.8 a	584 b	768 b	1.40	0.55	3.3 a	3.7 a
	2	17.8 b	21.5 b	39.3 a	2,112 a	4,474 a	1.60	0.88	2.7 b	3.2 ab
	3	2.7 c	5.6 c	8.3 b	137 b	3,531 a	1.37	0.72	1.5 c	3.0 b
Mi:Rr	0:0	16.5	18.4 a	34.9	—	—	—	—	—	—
Ratio	100:0	16.6	10.6 ab	27.3	1,031	—	1.00	—	2.8	3.6
	75:25	15.7	9.6 b	25.3	1,285	1,329 b	1.40	0.37 b	2.4	3.2
	50:50	24.1	12.4 b	36.5	713	2,421 b	2.02	0.53 ab	2.4	3.2
	25:75	16.5	12.0 b	28.5	750	2,886 b	1.39	0.96 a	2.4	3.1
	0:100	15.4	14.8 ab	30.2	—	5,061 a	—	1.00 a	—	—
Source:										
Test		***	***	***	***	***	NS	NS	***	**
Mi:Rr Ratio		NS	***	NS	NS	***	NS	**	NS	NS
Test × Mi:Rr ratio		NS	***	NS	NS	NS	NS	NS	*	NS

Means followed by the same letter do not differ (Tukey's HSD, $P \leq 0.05$).

[a] Severity is rated on a 0-5 scale where 0 = no galls and 5 = galls > 20 mm in diameter with major reduction in the number of feeder roots.

[b] Incidence is rated on a 0-4 scale where 0 = no galls and 5 = galls appearing on ≥ 76% of the root system.

*, **, and *** indicate significant differences at $P \leq 0.05$, 0.01, and 0.001, respectively. NS = means are not significantly different.

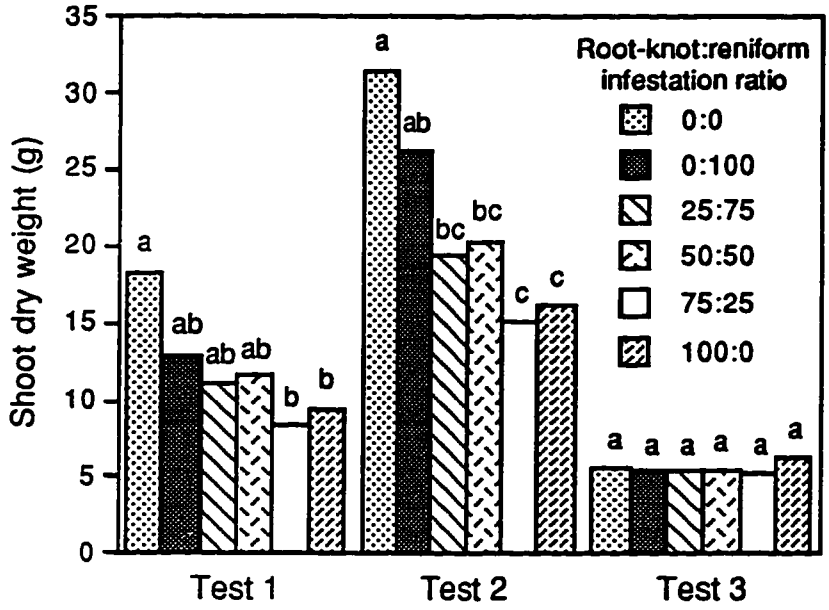

Fig. 2.3. Interaction between test and root-knot:reniform infestation ratio with regard to shoot dry weight 84-86 days after nematode infestation in three greenhouse tests; within each test, means separations are based on Tukey's HSD ($P \leq 0.05$).

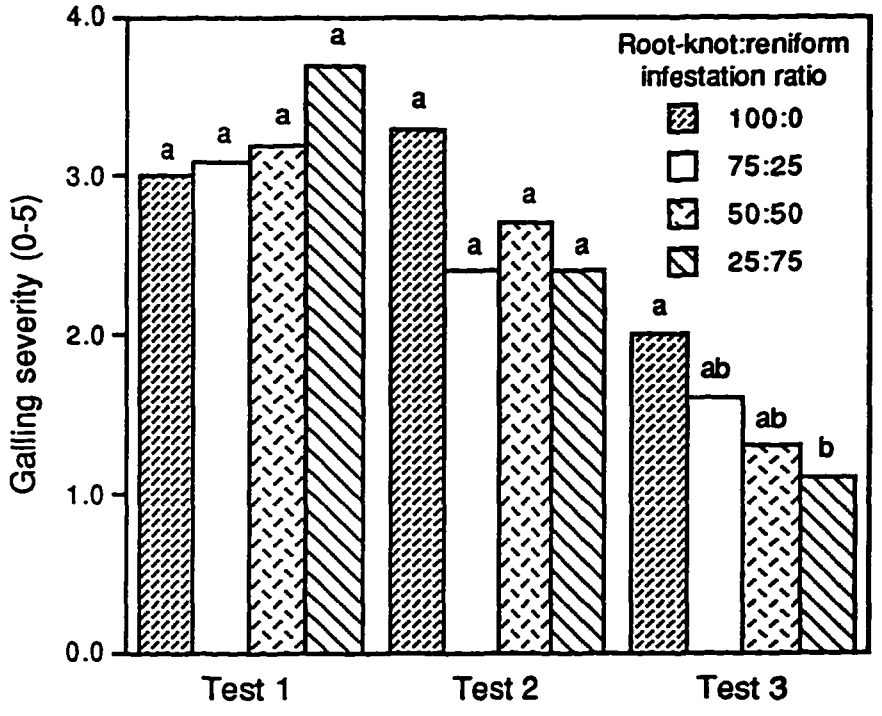

Fig. 2.4. Interaction between test and root-knot:reniform infestation ratio with regard to severity of galling induced 84-86 days after infestation by Meloidogyne incognita race 2 in three greenhouse tests; within each test, means separations are based on Tukey's HSD ($P \leq 0.05$).

galling observed at the 25:75 ratio was significantly lower than that seen at the 100:0 ratio in only one test.

Relative yield values calculated for root-knot and reniform populations did not differ between tests (Table 2.2). Root-knot relative yield values did not differ with respect to infestation ratio (Table 2.2). However, reniform relative yield values for the 75:25 ratio were lower than those for the 25:75 and 0:100 ratios (Table 2.2). No test by ratio interactions were detected for relative yield of either species, which allowed pooling of data from all three tests for regression analyses.

Discussion

An interrelationship exists between root-knot and reniform nematode on soybean cv. Davis, though the interpretation of the data depends largely on the analysis. ANOVA indicated suppression of reniform populations by concomitant colonization of the host by root-knot nematodes, with root-knot populations remaining unaffected. These results agree with the findings of Thomas and Clark (1983a), who documented a similar relationship between M. incognita race 1 and R. reniformis on sweet potato cvs. Centennial and Puerto Rico. However, our results are likely a combination of inoculum level effects and the biological interaction between the species. When the same data are analyzed using the replacement series approach, the effects of initial inoculum level are eliminated, and the focus shifts to the biological interaction between the species. In our studies, reniform yields were directly proportional to the input ratio, suggesting that the apparent suppression of this species was really an artifact of inoculum level. Instead, we detected a significant increase in root-knot relative yields whenever this species occurred in combination with reniform nematode. The mechanism for this increase, however, was not identified. It is possible that the stimulation of root-knot reproduction

is due to mechanisms operating at the genus level, though this hypothesis was not addressed in the current study.

The replacement series approach goes a step beyond ANOVA in detecting and defining relationships between organisms. This method eliminates potential confusion associated with inoculum levels. Replacement series deal with proportions rather than actual numbers, are less sensitive to nematode population fluctuations between seasons and environments, and often allow data from several experiments to be combined, thus strengthening statistical tests. If the hypothesized reference lines are representative of the species employed in the study, as they were in this research, then any significant deviation from the reference lines will be related to a biological phenomenon rather than initial inoculum level. The inherent reproductive capacities of the species under study should be compared with the hypothesized reference lines to be sure that these lines are appropriate. If the species in question do not conform to these lines, the replacement approach may not be applicable.

There are several potential constraints to using a replacement series approach. The results and their interpretation are density-dependent. Relationships detected at one initial community density may change dramatically if the density is altered. Care should be taken to choose one or more densities within the naturally occurring range for the species in question. For studies involving phytoparasitic nematodes, it is common to compare pathogen treatments with noninoculated controls to document the effects of the pathogen on parameters such as plant weight, yield, or symptom expression. Replacement series do not include such controls. It may not be possible to document the effects of a particular treatment on plant or symptom parameters without using ANOVA. In this research, the negative impact of Dpc on root-knot relative yields and galling was not evident from the replacement series analysis. Finally, because relative

yield values are ratios of final to initial levels of a pathogen or its propagules, they do not lend themselves to calculations involving plant or symptom data. We believe that employing a replacement series in addition to ANOVA will allow a more thorough examination of the biological system than either approach used alone.

Most significant interactions occur when pathogens attack the same region of the host (Powell, 1963, 1971). The fungus in this study colonizes the stem, a portion of the plant widely separated from the roots which harbor the nematode, so any impact of the fungus on the nematodes or their interactions would have to be indirect. The lower relative nematode yields and reduced galling on plants colonized by Dpc constitute the first report of this fungus inhibiting M. incognita race 2. A similar phenomenon was reported by Russin et al. (1989), who documented reductions in Heterodera glycines Ichinohe populations developing on Dpc-colonized soybean cv. Bragg. We were not able to determine whether the inhibition of root-knot nematode resulted from fungal damage to host tissue, a change in host physiology induced by colonization with Dpc, induced resistance, or other unidentified factors. The relationship between root-knot and reniform nematode appears to be independent of host colonization by Dpc, because this fungus did not differentially alter either the population sizes or the relative yields of either nematode species.

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CHAPTER 3

A GRINDING METHOD FOR EXTRACTION OF ROOT-ASSOCIATED NEMATODES

Introduction

Competition studies involving phytoparasitic nematodes require accurate enumeration of each species from both the soil and root microenvironments. Changes in soil populations may reflect migrations out of or into root systems rather than actual changes in reproduction.

The number of eggs extracted using the stirring procedure described by Hussey and Barker (1973) is often included in population assessments of phytoparasitic nematodes (Hirunsalee et al., 1995; Kirkpatrick et al., 1995; Montalvo and Esnard, 1994; Robbins et al., 1994; Sankaralingam and McGawley, 1994; Starr and Black, 1994; Walters and Barker, 1994; Weibelzahl-Fulton et al., 1996). While this provides a more accurate population assessment for monospecific studies, it is not feasible in studies involving two or more species because they can not be readily distinguished based on egg morphology. Root-knot nematodes (Meloidogyne incognita (Kofoid & White) Chitwood race 2) can infect and exist as endoparasites beginning at J2 and are less likely to be recovered from soil samples than are reniform nematodes (Rotylenchulus reniformis Linford & Oliviera), which do not infect until J4. To define the relationship between root-knot and reniform nematode on soybean, a rapid method to liberate vermiform and swollen individuals from root tissues was required.

Many of the protocols for quantifying root-associated nematodes have limitations. Baermann funnels (Agrios, 1988) have been utilized to recover migratory nematodes from root tissue (MacGuidwin and Forge, 1991; Vrain, 1977) and soil (Kotcon et al., 1987; Robinson and Heald, 1989, 1991), but root-knot and reniform females are sedentary. Clearing root tissue and staining endoparasitic nematodes (Byrd et al., 1983) also has been used routinely (Halbrendt et al., 1992; Hirunsalee et al., 1995; Zhang and Schmitt, 1995). However, staining may mask anatomical details

required for identification of species or development stages. Additionally, it is quite difficult to accurately count individuals in galled tissue due to the thickness of the root. Dislodging nematodes through enzymatic maceration of host tissue has been considered an effective extraction method (Araya and Caswell-Chen, 1993; Dickson et al., 1970; Dropkin et al., 1960; Hussey, 1971; Kaplan and Davis, 1990). However, this technique can be time-consuming, as constant agitation of plant material in the enzyme solution is required for a minimum of 5 hours and may take as long as 36 hours.

Physical maceration of host tissue in a blender has been used to liberate eggs of Meloidogyne incognita (McClure et al., 1973) and Pratylenchus penetrans (Dunn, 1973). Physical maceration used in conjunction with centrifugation was considered a reliable extraction method for all developmental stages of Tylenchulus semipenetrans (Greco and D'Addabbo, 1990). The primary objective of this investigation was to establish a similar procedure to liberate vermiform and swollen stages (nematodes) of root-knot and reniform nematode through mechanical disruption of the root tissue. A secondary objective was to compare the efficiency of the devised procedure to that of the standard egg extraction procedure (Hussey and Barker, 1973) for recovery of eggs.

Materials and Methods

General procedures: Tomato (Lycopersicon esculentum L. 'Rutgers') and soybean (Glycine max (L.) Merrill 'Davis') plants were grown in a greenhouse, where temperatures ranged from 22-35°C. Supplemental incandescent and fluorescent lighting (ca. 260 $\mu E \cdot m^{-2} \cdot sec^{-1}$) provided a minimum of 14 hours of light daily. These studies utilized 15-cm-diam. clay pots that contained approximately 1.6 kg of a soil mixture composed of three parts fumigated (67% methyl bromide, 33% chloropicrin) Convent silt loam soil (Aeric Fluvaquent, coarse-silty, mixed, non-acid, thermic) and two parts autoclaved sand.

Soybean seeds were treated with a commercial preparation of Bradyrhizobium japonicum (Kirchner) Jordan (Nitragin; The Nitragin Co., Milwaukee, WI) and sown in flats. Seedlings of uniform size were selected when plants were at growth stage V1 (Fehr et al., 1971), and transplanted to each test pot. Tomato seeds were sown in flats and 3- to 4-week-old seedlings were transplanted. Plants were fertilized three days after transplanting with 120 ml of a 23-19-17 N-P-K fertilizer solution (RapidGro; Chevron Chemical Co., San Ramon, CA). Plants received approximately 26 ppm N, 20 ppm P, and 33 ppm K.

Root-knot and reniform nematode populations were derived from single egg masses and maintained on tomato cv. Rutgers in a greenhouse. Inoculum consisted of 1,000 vermiform nematodes obtained from soil by hand-sieving and centrifugal flotation (Jenkins, 1964). Soil in each pot was infested by pipetting nematodes suspended in tap water into two depressions made in the soil. Each depression was 1 cm in diameter and 4 cm deep. After infestation, the depressions were filled with additional fumigated soil.

At harvest, root systems were freed from soil by gentle washing in tap water. Root systems were cut into 2.5-cm segments and subsamples were selected at random for nematode extraction by stirring (Hussey and Barker, 1973) or grinding in 60 ml of water or 0.5% NaOCl. Root tissue was ground at maximum speed in a Waring commercial blender (model 31BL42) fitted with a 500-ml pulverizing container. The contents of the beaker (stirred treatments) or pulverizing container (ground treatments) were poured onto nested 75- and 25-μm-pore sieves and nematodes and eggs were counted.

Experiment 1: The objectives of this experiment were to optimize a grinding method for extracting root-knot and reniform nematodes from tomato and soybean roots and to compare the efficiency of stirring and grinding methods for extracting eggs. The

experiment was conducted twice. Root-knot and reniform nematodes were introduced into the soil in pots containing three seedlings of uniform size. Monospecific populations were allowed to develop for 59 (Test 1) or 64 (Test 2) days. At harvest, the root tissue from all pots sharing the same nematode and host was combined and subsampled (1.5 g). Within each nematode species and host, treatments were combined in a factorial arrangement and assigned in a completely randomized design. Treatments were: method (stir, grind), carrier (water, 0.5% NaOCl), and duration (1X, 2X). Durations were 5 sec and 5 min for the 1X level in ground and stirred treatments, respectively. Treatments were replicated four times. Data were analyzed using analysis of variance (ANOVA) and contrasts ("Fit Model" module of SAS JMP version 3.0) (SAS Institute, 1994). Interactions which were significant in two or more test and host combinations are presented as figures; those which occurred only once are described in the text.

Experiment 2: Results from Experiment 1 indicated that root-associated populations of reniform nematode were consistently small. Therefore, Experiment 2 employed only root-knot nematode. The objective was to compare the optimized grinding procedure with the standard stirring extraction method for liberating nematodes and eggs from soybean roots of older plants. The experiment was conducted twice. One soybean seedling was transplanted into each of ten test pots. Root-knot nematodes were introduced into the soil in each pot and populations were allowed to develop for 91 (Test 1) or 93 (Test 2) days. At harvest, two subsamples (2 g) were taken from the root system in each pot and either stirred in 0.5% NaOCl for 10 min or ground in 0.5% NaOCl for 10 sec. Data were analyzed using analysis of variance (ANOVA) ("Fit Model" module of SAS JMP version 3.0) (SAS Institute, 1994).

Results

Experiment 1: Root-associated populations of reniform nematode were considerably smaller than populations of root-knot nematode on both tomato and soybean (Tables 3.1 and 3.2). Egg and nematode counts for root-knot nematode were higher for tomato than for soybean, whereas the two hosts yielded similar numbers of reniform nematodes and eggs (Tables 3.1 and 3.2). Numerous test by treatment interactions were detected in the initial analyses, so each test was analyzed independently.

Recovery of reniform nematodes is summarized in Table 3.1. Stirring and grinding treatments liberated equivalent numbers of nematodes. Carrier and duration of treatment did not influence the number of nematodes recovered. A carrier by duration interaction was detected on tomato in Test 1. When treatments were processed in water, more nematodes were liberated from treatments of longer duration. Treatments processed in 0.5% NaOCl liberated equivalent numbers of nematodes from the 1X and 2X durations.

Recovery of reniform eggs is summarized in Table 3.1. Stirring liberated significantly more intact eggs than grinding from tomato roots in Test 1. For all other test and host combinations, the methods liberated equivalent numbers of intact eggs. Carrier and duration of treatment did not influence the number of eggs recovered. Examination of a method by carrier interaction on tomato in Test 1 showed that 0.5% NaOCl increased recovery of eggs only for stirred treatments.

Recovery of root-knot nematodes is summarized in Table 3.2. Grinding liberated more nematodes than stirring in two of the four test and host combinations. More nematodes were recovered from treatments processed in 0.5% NaOCl for tomato

Table 3.1. Effect of treatment method, duration, and carrier on extraction of vermiform or swollen individuals (nematodes) or eggs of Rotylenchulus reniformis from tomato or soybean roots.

Treatment	Level	Nematodes/g fresh root tissue				Eggs/g fresh root tissue			
		Soybean		Tomato		Soybean		Tomato	
		Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
Method (M)	Stir	8	3	15	1	40	13	38	3
	Grind	4	6	12	4	14	11	17	3
Carrier (C)	Water	6	4	16	1	19	7	24	3
	0.5% NaOCl	5	4	12	4	36	17	31	3
Duration (D)	1X[a]	6	3	11	4	38	17	34	6
	2X	6	6	17	1	17	7	21	0
Source:									
M		NS	NS	NS	NS	NS	NS	*	NS
C		NS	NS	NS	NS	NS	NS	NS	NS
D		NS	NS	NS	NS	NS	NS	NS	NS
M x C		NS	NS	NS	NS	NS	NS	**	NS
M x D		NS	NS	NS	NS	NS	NS	NS	NS
C x D		NS	NS	**	NS	NS	NS	NS	NS
M x C x D		NS	NS	NS	NS	NS	NS	NS	NS

[a] 1X = 5 sec or 5 min in grinding or stirring treatments, respectively.
Data are means of four replicates.
*, and ** indicate significant differences at $P \leq 0.05$ and 0.01, respectively. NS = means are not significantly different.

Table 3.2. Effect of treatment method, duration, and carrier on extraction of vermiform or swollen individuals (nematodes) or eggs of Meloidogyne incognita race 2 from tomato or soybean roots.

Treatment	Level	Nematodes/g fresh root tissue				Eggs/g fresh root tissue			
		Soybean		Tomato		Soybean		Tomato	
		Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
Method (M)	Stir	40	59	98	158	2,613	966	5,442	1,232
	Grind	78	226	124	420	738	427	1,897	686
Carrier (C)	Water	45	114	74	294	182	92	448	195
	0.5% NaOCl	72	171	148	284	3,169	1,301	6,892	1,723
Duration (D)	1X[a]	54	112	121	192	2,023	620	2,557	856
	2X	64	174	100	386	1,328	773	4,783	1,062
Source:									
M		NS	***	NS	***	***	*	***	NS
C		NS	NS	*	NS	***	***	***	***
D		NS	NS	NS	**	NS	NS	**	NS
M x C		NS	NS	NS	NS	***	*	***	*
M x D		NS	NS	NS	NS	*	NS	**	NS
C x D		NS	NS	NS	NS	*	NS	**	NS
M x C x D		NS	NS	NS	NS	*	NS	**	NS

[a] 1X = 5 sec or 5 min in grinding or stirring treatments, respectively.

Data are means of four replicates.

*, **, and *** indicate significant differences at $P \leq 0.05$, 0.01, and 0.001, respectively. NS = means are not significantly different.

roots in Test 1. More nematodes were recovered from treatments of longer duration for tomato roots in Test 2.

Recovery of root-knot eggs is summarized in Table 3.2. More intact eggs were recovered from stirred treatments than from ground treatments in three of the four test and host combinations. In all four test and host combinations, more eggs were recovered from treatments processed in 0.5% NaOCl. The longer treatment duration yielded more eggs from tomato in Test 1. A three-way interaction involving method, carrier, and duration was detected on both hosts in Test 1 (Fig. 3.1). For both hosts, duration of stirring influenced egg recovery only when treatments were processed in 0.5% NaOCl. On soybean, more eggs were recovered from treatments of shorter duration (Fig. 3.1). On tomato, more eggs were recovered from treatments of longer duration (Fig. 3.1). In addition, 0.5% NaOCl improved recovery of eggs from ground tomato roots only for treatments of longer duration (Fig. 3.1). Examination of a method by carrier interaction for both hosts in Test 2 showed that processing treatments in 0.5% NaOCl improved egg yield more from stirred treatments than from ground treatments (Fig. 3.2).

Experiment 2: Findings from Experiment 1 indicated that recovery of root-knot nematodes by grinding occasionally was improved by using 0.5% NaOCl instead of water as a carrier and by doubling the duration of grinding to 10 sec. This optimized grinding treatment yielded almost three times as many nematodes as stirring in 0.5% NaOCl for 10 min (Table 3.3). Again, stirring liberated more intact eggs than did grinding (Table 3.3).

Discussion

Grinding root tissue in 0.5% NaOCl for 10 sec gave optimum recovery of vermiform and swollen root-knot nematodes from tomato and soybean roots supporting

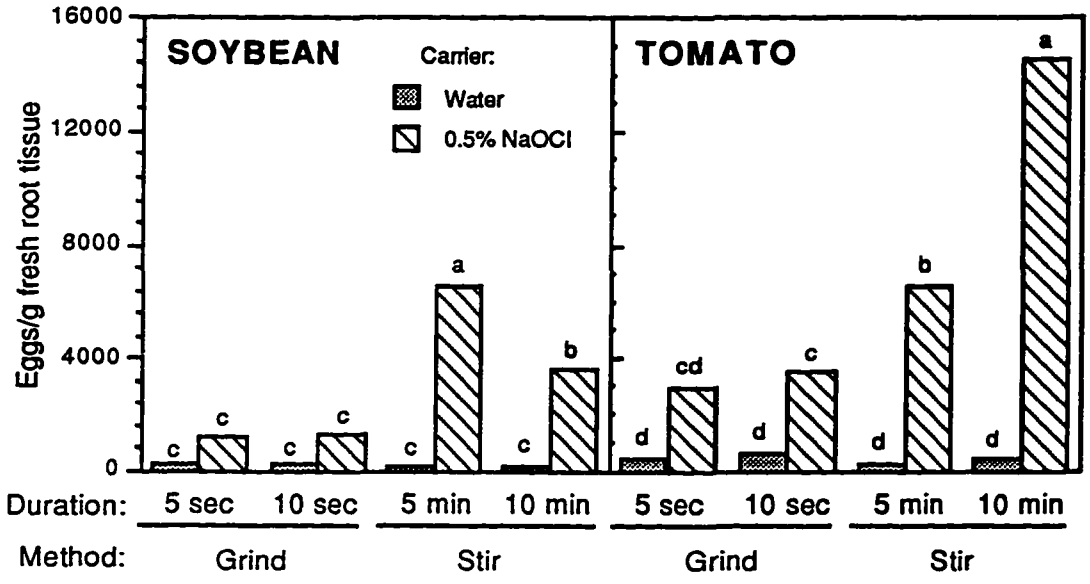

Fig. 3.1. Influence of treatment method, duration, and carrier on number of Meloidogyne incognita race 2 eggs recovered from soybean and tomato roots in Test 1. Data are means of four replicates. Within figures, means designated with the same letter do not differ ($P \leq 0.05$).

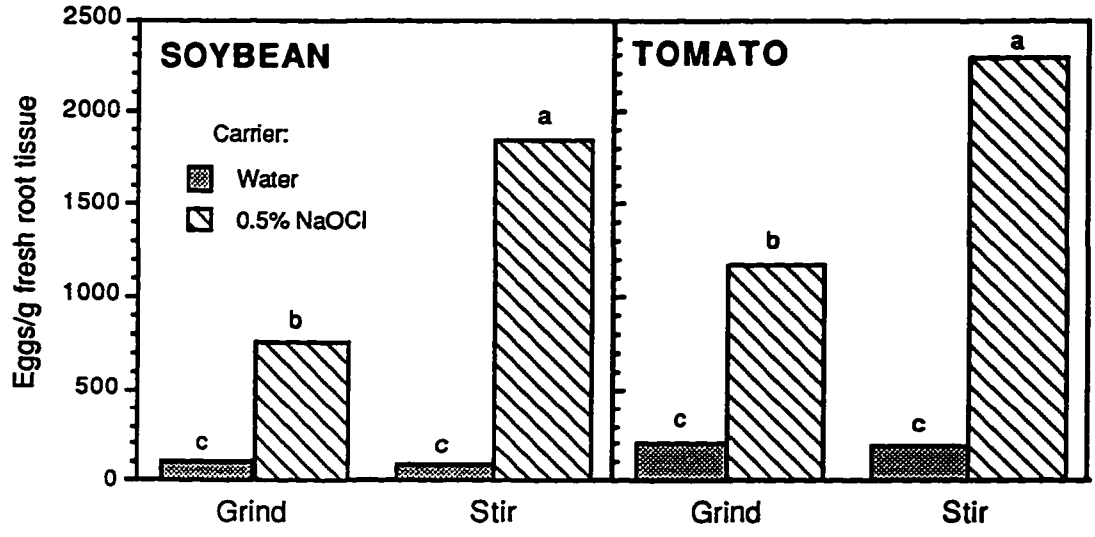

Fig. 3.2. Influence of treatment method and carrier on number of Meloidogyne incognita race 2 eggs recovered from soybean and tomato roots in Test 2. Data are means of four replicates. Within figures, means designated with the same letter do not differ ($P \leq 0.05$).

Table 3.3. Meloidogyne incognita race 2 nematodes and eggs recovered from Davis soybean roots by stirring for 10 min in 0.5% NaOCl or by grinding for 10 sec in 0.5% NaOCl.

Treatment	Level	Nematodes per g	Eggs per g
Test	1	122	1,779
	2	21	201
Method	Grind	107	573
	Stir	36	1,407
Source:			
Test		**	***
Method		*	*
Test x method		NS	NS

Data are means of 20 replicates in two tests.

*, **, and *** indicate significant differences at $P \leq 0.05$, 0.01, and 0.001, respectively. NS = means are not significantly different.

populations for approximately 60 days. This technique was applied to soybean roots thirty days older than those on which the procedure was first evaluated with consistent results. When used to extract reniform nematodes from host roots supporting comparatively lower populations, the technique did not improve recovery efficiency.

Araya and Caswell-Chen (1993) reported recovery of 194 - 706 juveniles and adult females of Meloidogyne javanica per gram of root tissue using enzymatic maceration, though numbers varied depending on the host plant species. The grinding procedures utilized in this study liberated 78 - 420 juveniles and adults of M. incognita per gram of tissue from two host species. The number of individuals recovered by grinding appears to be within range reported for enzymatic digestion, though caution must be used in drawing conclusions without data from experiments directly comparing these two methods using the same species of both nematode and host. However, the data suggest that the grinding technique is a quick, simple, and inexpensive alternative to the enzymatic maceration procedure for extracting juvenile and adult root-knot nematodes.

For extraction of eggs, the grinding procedure was not an improvement over the standard extraction procedure described by Hussey and Barker (1973) or the enzymatic digestion method described by Araya and Caswell-Chen (1993). The results of the current study confirmed the efficiency of stirring in 0.5% NaOCl, though the relative impact of the duration of this treatment was not consistent. A single sample may be processed by stirring for egg extraction and subsequently by grinding to liberate other developmental stages, providing a more complete assessment of root-associated nematode populations.

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CHAPTER 4

RELATIONSHIP BETWEEN *MELOIDOGYNE INCOGNITA* AND *ROTYLENCHULUS RENIFORMIS* AS INFLUENCED BY SOYBEAN GENOTYPE

Introduction

Root-knot (Meloidogyne incognita (Kofoid & White) Chitwood race 2) and reniform (Rotylenchulus reniformis Linford & Oliviera) nematodes are pathogenic to soybean (Glycine max (L.) Merrill) (Sinclair and Backman, 1989). These species share the same geographic and host ranges in Louisiana, where nematode damage reduced soybean yields 4-8% annually during 1988-1993 (Sciumbato, 1993; Wrather and Sciumbato, 1995).

In replacement series experiments, Erwin et al. (1995) and Stetina et al. (1997) showed increased root-knot nematode reproduction in the presence of reniform nematode. This was evidenced by relative yields for root-knot populations in soil that were significantly higher than predicted at all ratios where root-knot and reniform nematodes occurred together. Relative nematode yields for reniform populations in soil did not differ from predicted yields, which indicated no effect of root-knot nematode on reniform reproduction. These experiments, however, were limited to the soybean cultivar Davis, which is susceptible to root-knot nematode.

The objective of this research was to determine if the relationship between root-knot and reniform nematodes documented on a susceptible soybean cultivar was similar to that found on a resistant/tolerant soybean cultivar. A preliminary report has been published (Erwin et al., 1996).

Materials and Methods

General procedures: Two experiments were conducted in a greenhouse where temperatures ranged from 22-35°C. Supplemental incandescent and fluorescent lighting (ca. 260 $\mu E \cdot m^{-2} \cdot sec^{-1}$) provided a minimum of 14 hours of continuous light daily. These studies utilized 15-cm-diam. clay pots that contained approximately 1.6 kg of a soil mixture composed of three parts fumigated (67% methyl bromide, 33%

chloropicrin) Convent silt loam soil (Aeric Fluvaquent, coarse-silty, mixed, nonacid, thermic) and two parts autoclaved sand.

Two experiments were conducted in microplots. Each microplot consisted of a 30-cm-diam. clay pot that contained approximately 15 kg of fumigated (32.7% sodium methyldithiocarbamate, 67.3% inert ingredients; 18.8 ml fumigant in 882 ml water per pot) Mhoon silt loam soil (Typic Fluvaquent, fine-silty, mixed, nonacid, thermic). Microplots were set into the ground to the depth of the pot rim and spaced 1 m apart. The entire microplot area was sheltered by a polyethylene-covered quonset hut frame, open at both ends, and covered with black shade cloth. Plants in microplots received 516 $\mu E \cdot m^{-2} \cdot sec^{-1}$ of light (approximately 30% of full sunlight). Supplemental lighting was not used in the microplot area.

Soybean cv. Davis or Buckshot 66 seeds were treated with a commercial preparation of Bradyrhizobium japonicum (Kirchner) Jordan (Nitragin; The Nitragin Co., Milwaukee, WI) and sown in flats. Seedlings of uniform size were selected when plants were at growth stage V1 (Fehr et al., 1971), and a single seedling was transplanted to the center of each test pot for greenhouse tests or to a 10-cm square cell pack for microplot tests. Plants were fertilized three days after transplanting with 120 ml of a 23-19-17 N-P-K fertilizer solution (RapidGro; Chevron Chemical Co., San Ramon, CA). Plants received approximately 26 ppm N, 20 ppm P, and 33 ppm K.

Root-knot and reniform nematode populations were derived from single egg masses and maintained on tomato (Lycopersicon esculentum L. 'Rutgers') in a greenhouse. Inoculum consisted of vermiform nematodes obtained from soil by hand-sieving and centrifugal flotation (Jenkins, 1964). Soil in each pot was infested with the required number of each species by pipetting nematodes suspended in tap water into two depressions made in the soil. Tap water was pipetted into depressions in control pots.

Each depression was 1 cm in diameter, 4 cm deep, and 5 cm from the base of the stem on opposite sides of the plant. After infestation, the depressions were filled with additional fumigated soil.

In greenhouse tests, pots remained undisturbed until harvest. In microplot tests, the plant and soil from the cell pack were transferred 10 days after infestation into a depression of comparable size made in the microplot soil. Pots then remained undisturbed until harvest.

At the end of each experiment, five soil cores 2.5 cm in diameter and 30 cm deep were collected from each pot, mixed thoroughly, and subsampled (150 g). Nematodes were extracted by hand-sieving and centrifugal flotation. Numbers of juveniles, males, vermiform females, and swollen females collected on a 38-μm-pore sieve were recorded for each species.

Plant stems were cut at the soil surface and the root-soil mass was removed from each pot. Root systems were freed from soil by gentle washing in tap water. Root-knot gall severity was rated according to the following scale: 0 = no galls, 1 = galls less than 3 mm in diameter with no reduction in the number of feeder roots, 2 = galls 3-10 mm in diameter with no reduction in the number of feeder roots, 3 = galls 10-20 mm in diameter with no or slight reduction in the number of feeder roots, 4 = galls >20 mm in diameter with moderate reduction in the number of feeder roots, and 5 = galls >20 mm in diameter with major reduction in the number of feeder roots. Root-knot gall incidence was rated according to the following scale: 0 = no galls, 1 = galls confined to 25% or less of the root system, 2 = galls appearing over 26-50% of the root system, 3 = galls appearing over 51-75% of the root system, and 4 = galls appearing on 76% or more of the root system.

Nematodes were extracted from a subsample (2 g) removed at random from each root system. Root tissue was combined with 60 ml of 0.5% NaOCl and ground for 10 sec at maximum speed in a Waring commercial blender (model 31BL42) fitted with a 500-ml stainless steel pulverizing container. The slurry was poured onto nested 75- and 25-µm-pore sieves, and vermiform and swollen individuals of each nematode species were counted. Eggs collected on the 25-µm-pore sieve could not be identified to species, so egg counts were not included in population totals.

Replacement series experiments: The relationship between root-knot and reniform nematode was examined on soybean cv. Davis (susceptible to both root-knot and reniform nematode) and Buckshot 66 (resistant to root-knot, susceptible to reniform nematode). Experiments on each cultivar were conducted twice, i. e., in both greenhouse and microplot. All four experiments were established using a randomized complete block design with five (microplot) or ten (greenhouse) replications. Root-knot and reniform nematodes were introduced alone or in combination at an initial community density of 1,000 individuals per pot when plants reached growth stages V2 - V3. Soil was not infested or nematodes were introduced at one of the following root-knot:reniform ratios: 100:0, 75:25, 50:50, 25:75, and 0:100. Experiments were terminated 91-93 days after nematode infestation (R4 or R5 in greenhouse tests, R6 in microplot tests). At harvest, plants were divided into root and shoot portions by cutting the stem at the soil line. Soybean root and shoot dry weights (after drying at 70°C for 4 days) were recorded after galling assessment and collection of tissue samples for nematode extraction. Soil samples were processed and nematodes counted. Relative nematode yields were based on the total number of nematodes of each species extracted from soil and roots, expressed per gram of dry root tissue. For these experiments, relative yield was calculated by dividing the number of nematodes of a species recovered

from mixed culture by the number of nematodes of the same species recovered from nonmixed culture (Stetina et al., 1997).

Data presentation and analyses: To examine the relationship between root-knot and reniform nematodes, differences between the predicted relative yield lines (representing equal interspecific and intraspecific competition) defined by the replacement series model (de Wit, 1960; de Wit et al., 1966) and the relative nematode yield lines plotted using calculated relative nematode yield values were determined by lack-of-fit regression ("Fit Model" module of SAS JMP version 3.0) (SAS Institute, 1994). Paired t-tests ("Fit Y by X" module of SAS JMP version 3.0) (SAS Institute, 1994) were used to determine at which ratio(s) the predicted and calculated relative nematode yield values differed. Plant weights were analyzed using analysis of variance (ANOVA), Fisher's protected LSD, and orthogonal polynomial contrasts ("Fit Model" and "Fit Y by X" modules of SAS JMP version 3.0) (SAS Institute, 1994). Galling indices for plants inoculated with root-knot were examined using orthogonal polynomial contrasts ("Fit Model" module of SAS JMP version 3.0) (SAS Institute, 1994).

Results

Nematodes, alone or together, did not impact shoot or root weight of Davis or Buckshot 66 in greenhouse tests (Fig. 4.1A-D). In microplot tests, Davis shoot weights were lowest on plants inoculated with high levels (100:0, 75:25) of root-knot nematode (Fig. 4.1A). Shoot weights increased in a linear fashion ($t = 4.35$, $P > |t| = 0.0008$) as the proportion of this nematode in the inoculum decreased. Plants inoculated with mixtures of root-knot and reniform nematodes had heavier roots than the noninoculated control (Fig. 4.1B). A quadratic relationship ($t = -3.06$, $P > |t| = 0.0090$) was detected among all inoculated treatments, with heavier root systems recovered from plants colonized by mixtures of nematodes. When root-knot nematode

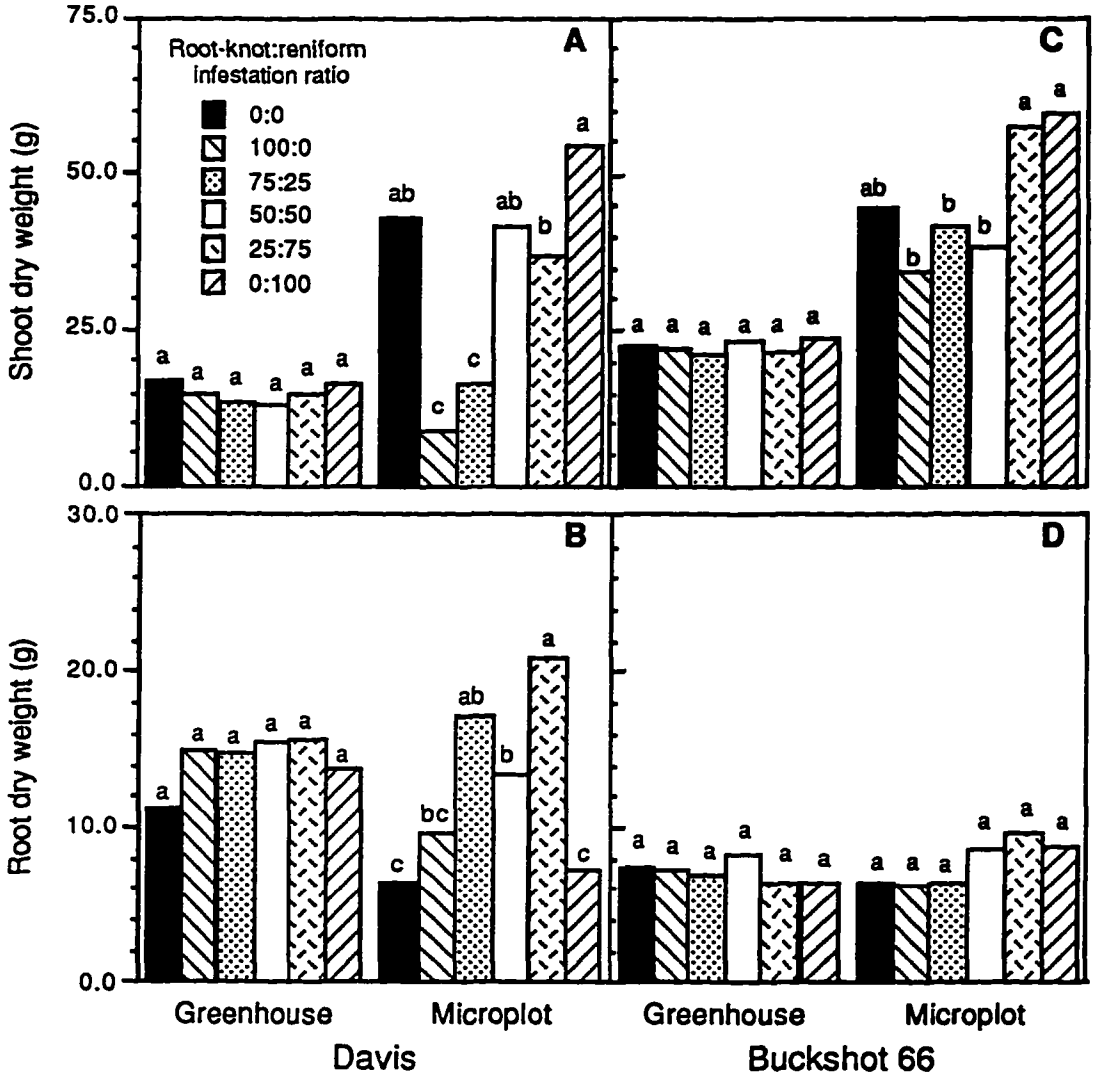

Fig. 4.1. Effect of Meloidogyne incognita race 2 (root-knot) and Rotylenchulus reniformis (reniform) on dry weight of the soybean cultivars Davis (susceptible to both species) and Buckshot 66 (resistant to root-knot, susceptible to reniform) in greenhouse (10 replications) and microplot (5 replications) tests. Within each parameter, cultivar, and location, means with the same letter do not differ (Fisher's protected LSD, $P \leq 0.05$). A) Shoot dry weight on Davis. B) Root dry weight on Davis. C) Shoot dry weight on Buckshot 66. D) Root dry weight on Buckshot 66.

was included in the inoculum, root weight increased in a linear manner ($t = -2.47$, $P > |t| = 0.0357$) as the level of this nematode decreased. In the microplot, Buckshot 66 plants inoculated with low levels (25:75, 0:100) of root-knot nematode had heavier shoots than plants inoculated with moderate to high levels of this species, though weights in both groups did not differ from noninoculated controls (Fig. 4.1C). Root dry weights were not influenced by nematode colonization at any ratio on Buckshot 66 in the microplot test (Fig. 4.1D). Orthogonal polynomial contrasts did not reveal any trends in shoot or root weight related to nematode infestation on Buckshot 66.

Incidence and severity of galling were generally greater on Davis than on Buckshot 66 in both greenhouse and microplot tests (Fig. 4.2A-D). Galling was so severe on Davis that feeder roots were almost completely absent in the microplot test. On Davis, orthogonal polynomial contrasts revealed a cubic relationship between the proportion of root-knot in the inoculum and gall incidence ($t = -2.33$, $P > |t| = 0.0378$) and severity ($t = -2.85$, $P > |t| = 0.0146$) in the microplot test (Fig. 4.2A,B). For both indices, minimum values were associated with the 50:50 ratio. In the greenhouse, gall incidence decreased linearly in proportion to lower levels of root-knot nematode in the inoculum ($t = 3.03$, $P > |t| = 0.0053$) (Fig. 4.2A). No relationship between nematode ratio and severity of galling was detected on Davis in the greenhouse test (Fig. 4.2B). On Buckshot 66, no relationships between nematode ratio and either incidence or severity of galling were detected in greenhouse or microplot tests (Fig. 4.2C,D).

On Davis, relative root-knot yields in the greenhouse test were significantly higher than predicted ($F = 4.26$, $P = 0.0099$), notably at the 50:50 ratio. Reniform relative nematode yields were not influenced by colonization of the same host by root-knot nematode ($F = 0.60$, $P = 0.6171$) (Fig. 4.3A). In the microplot test, both root-knot and reniform relative nematode yields were lower than predicted (for root-knot,

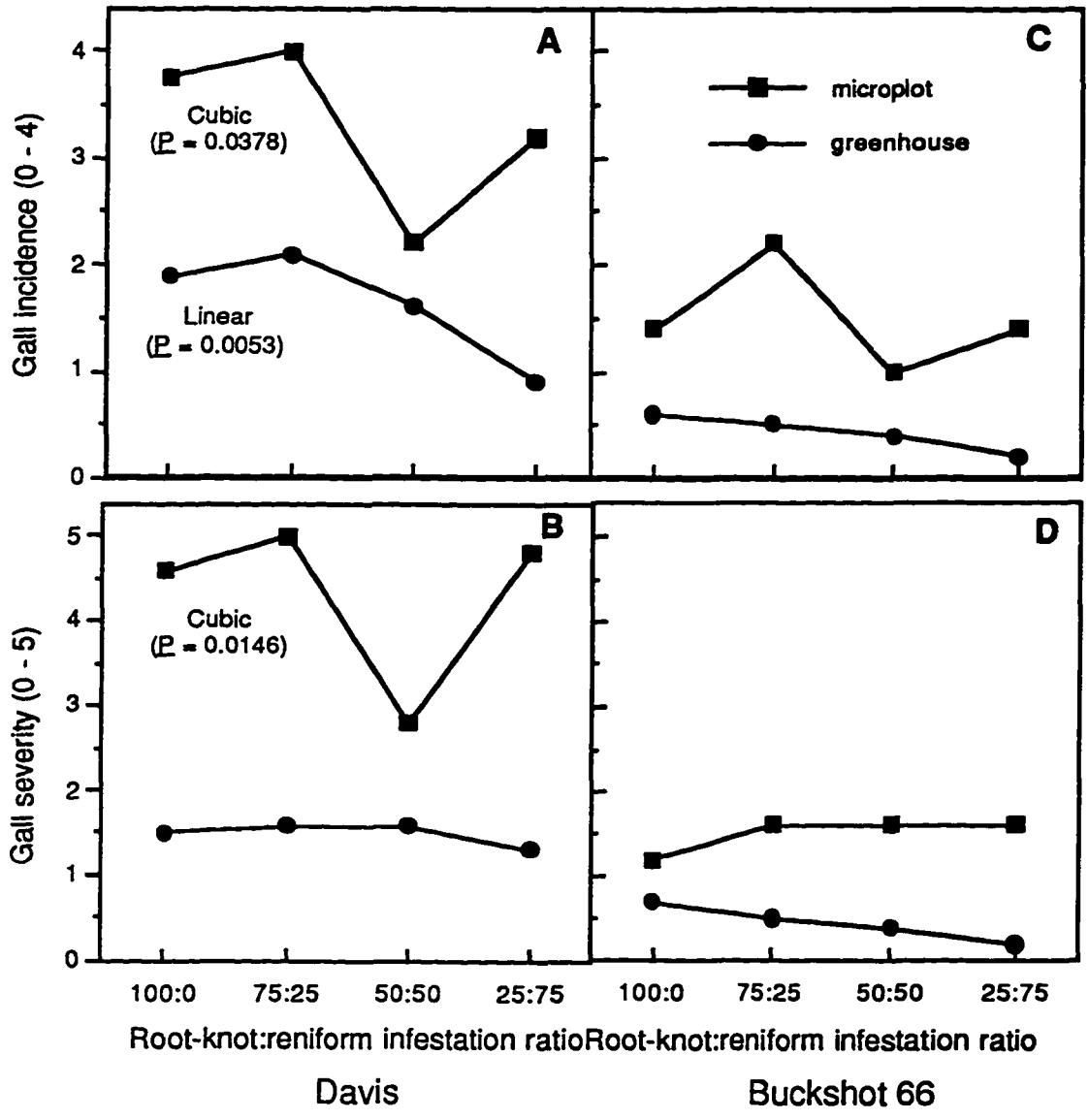

Fig. 4.2. Relationships between proportion of Meloidogyne incognita race 2 (root-knot) in the inoculum and severity and incidence of galling on the soybean cultivars Davis (susceptible to root-knot) and Buckshot 66 (resistant to root-knot) 91-93 days after nematode infestation. Incidence is rated on a 0 - 4 scale where 0 = no galls and 4 = galls appearing on 76% or more of the root system. Severity is rated on a 0 - 5 scale where 0 = no galls and 5 = galls >20 mm in diameter with major reduction in the number of feeder roots. The nature of the relationship and $P > |t|$ are noted where significant. A) Incidence on Davis. B) Severity on Davis. C) Incidence on Buckshot 66. D) Severity on Buckshot 66.

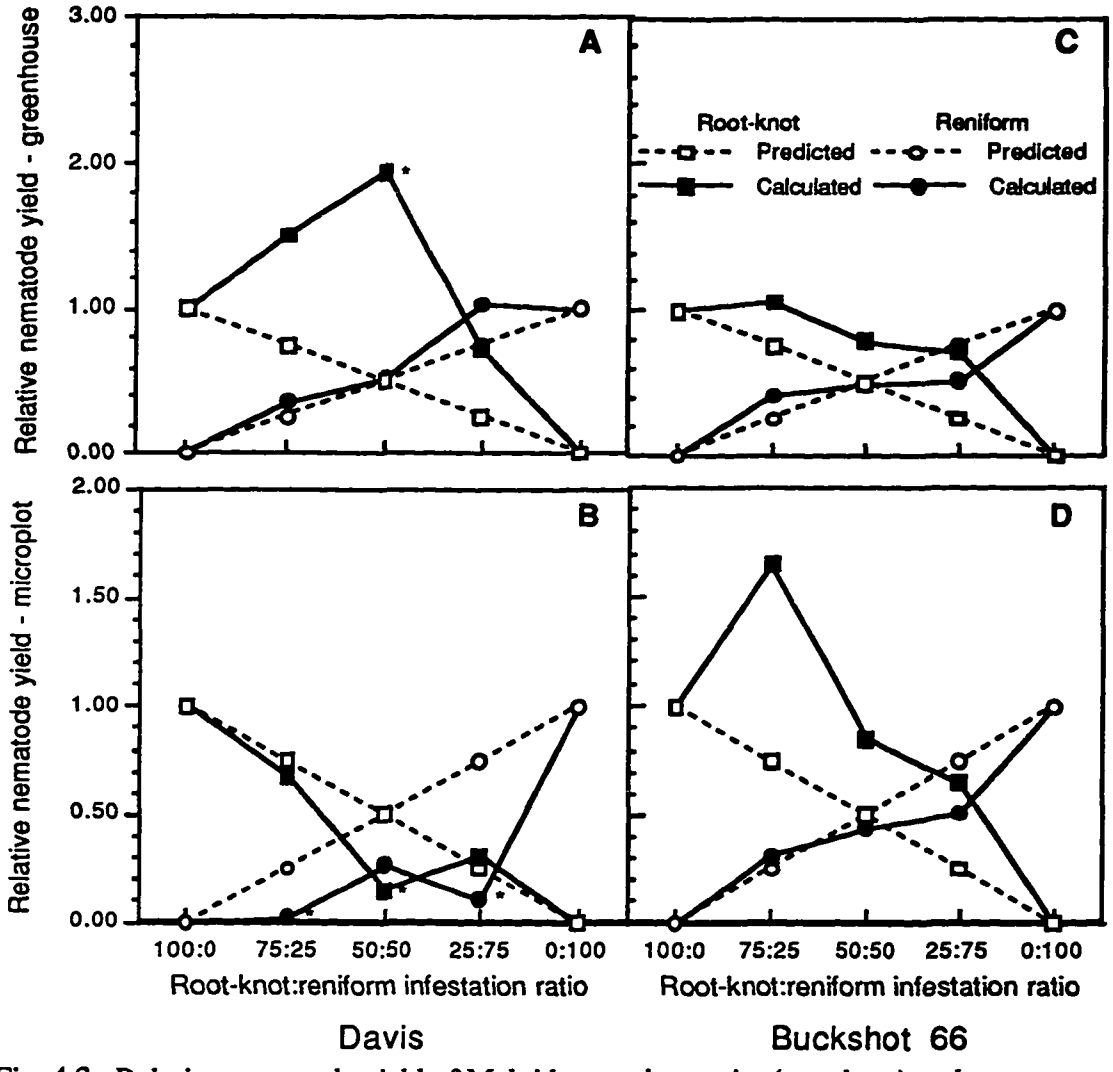

Fig. 4.3. Relative nematode yield of Meloidogyne incognita (root-knot) and Rotylenchulus reniformis (reniform) 91-93 days after infestation on the soybean cultivars Davis and Buckshot 66 in greenhouse and microplot tests. Calculated values that differ significantly ($P \leq 0.05$) from predicted relative nematode yields are indicated with an asterisk to the right of the calculated mean. A) Davis, greenhouse. B) Davis, microplot. C) Buckshot 66, greenhouse. D) Buckshot 66, microplot.

$F = 3.28$, $P = 0.0422$; for reniform, $F = 12.80$, $P \leq 0.0001$) (Fig. 4.3B). Significant reductions in relative nematode yields occurred at the 50:50 ratio for root-knot nematode and at the 75:25 and 25:75 ratios for reniform nematode (Fig. 4.3B). On Buckshot 66, no significant differences were detected between calculated and predicted relative nematode yields for either species in greenhouse or microplot tests (Fig. 4.3C,D).

Discussion

The ability of one nematode population to influence the reproduction of a second nematode population is a key factor affecting interspecific competition (Eisenback, 1993). The results of the current study closely agree with those of Erwin et al. (1995) and Stetina et al. (1997), who first reported that colonization of the soybean cultivar Davis by reniform nematodes consistently increased root-knot nematode reproduction under greenhouse conditions. The stimulatory effect of reniform nematode on root-knot nematode is not an isolated example of enhanced reproduction by nematodes in coexistence. Increased reproduction of: Belonolaimus longicaudatus in the presence of Hoplolaimus galeatus on cotton (Yang et al., 1976); Hoplolaimus columbus in the presence of M. incognita or Scutellonema brachyurum on cotton (Kraus-Schmidt and Lewis, 1981); Criconemella xenoplax in the presence of Meloidogyne hapla on grape (Santo and Bolander, 1977; Pratylenchus brachyurus in the presence of M. incognita on tobacco cv. NC 2512 (Johnson and Nusbaum, 1970); and Paratrichodorus minor in the presence of P. brachyurus on soybean (Johnson and Nusbaum, 1968) have been documented. Mutual stimulation of P. minor and Pratylenchus zeae on corn (Johnson and Nusbaum, 1968) and H. columbus and S. brachyurum on cotton (Kraus-Schmidt and Lewis, 1981) have also been reported.

The specific mechanisms contributing to stimulatory relationships have not been determined. Colonization by one species may alter the amount or composition of root

exudates, making the roots more attractive to individuals of another species. Concomitant infections may alter the physiological reactions of the host which lead to resistance (Griffin, 1980), or they may alter levels of translocatable substances that benefit one or both nematode species or impact host resistance (Eisenback, 1983). Further studies are required to elucidate the mechanism behind the stimulatory effect of reniform nematode on root-knot nematode on Davis soybean in this system.

The suitability of a host species or genotype can affect the ecological and etiological associations among nematode species. The relationship between M. incognita and R. reniformis is dramatically influenced by the biology of the host plant. Enhanced reproduction was noted on the root-knot-susceptible cultivar Davis, while nematode yield was directly proportional to the level of M. incognita in the inoculum on the root-knot-resistant cultivar Buckshot 66. The influence of the host is evident in other nematode-host-nematode systems as well. Inoculation with M. incognita inhibited subsequent penetration of tomato roots by P. brachyurus but stimulated penetration of cotton roots by this species (Gay and Bird, 1973). Johnson and Nusbaum (1970) documented inhibitory, neutral, and stimulatory associations between M. incognita, P. brachyurus, and M. hapla on tobacco, which differed in both nature and magnitude depending on the host cultivar. The associations were species-specific, as M. incognita did not have the same impact on P. brachyurus as another root-knot species, M. hapla. In split-root experiments, Eisenback (1983) reported that inoculation of root-knot-resistant tobacco with Meloidogyne arenaria or M. hapla masked the resistance of that cultivar to M. incognita race 1 when this species was subsequently introduced. Griffin (1980) found that infection by Ditylenchus dipsaci reduced the resistance of the alfalfa cultivar Vernal 298 to M. hapla. Finally, McGawley and Winchell (1987) reported that

galling of soybean induced by a combination of M. incognita and Meloidogyne javanica was significantly greater than when either species was inoculated independently.

The genotype of the soybean host influenced the relationship between root-knot and reniform nematode. In the greenhouse test, the susceptibility of Davis to root-knot nematode increased when reniform nematode colonized the same host, as evidenced by higher relative root-knot nematode yields. In the microplot test, enhanced root-knot reproduction probably began early in the season, resulting in severe galling, destruction of feeder roots, and the dramatic population declines reflected in the relative nematode yields seen 91-93 days after infestation for both species. The reduction in shoot weight seen in the microplot test may also be attributable to the significant damage caused by the large root-knot population. The microplot infestation method may have concentrated high numbers of nematodes in a small area, thereby increasing the potential of individual nematodes to locate and infect roots at the beginning of the experiment. In addition, the microplot environment was less subject to temperature and moisture fluctuations which can impact nematode population development than was the greenhouse environment. A combination of these factors may have contributed to the apparent increase in susceptibility to root-knot nematode on Davis in the microplot. In spite of these factors, enhanced susceptibility to root-knot nematode was not observed on the cultivar Buckshot 66, which remained tolerant to this species even when colonized by reniform nematodes. This suggests that the inherent resistance of the host is the primary factor determining the nature of the relationship between root-knot and reniform nematode.

Relationships defined on one host may be quite different on other cultivars or host species. However, host suitability is not the only factor capable of influencing the ecological association among nematode species. Edaphic factors such as soil texture, soil moisture, and temperature; nematode population densities, timing and method of

nematode inoculation, pesticides applied, and the influence of other biological entities within the system may alter nematode relationships. To fully document the interrelationships between two nematode species, they should be evaluated under a range of biotic and abiotic conditions.

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CHAPTER 5

SUMMARY AND CONCLUSIONS

The replacement series approach was a sensitive and useful tool for evaluating the relationship between phytoparasitic nematodes, because biological phenomena are not obscured by inoculum dosage effects. The replacement series approach should be easily adapted to other nematode systems, provided that reference lines indicative of expected relative nematode yields are used. This approach is not as easily influenced by environmental and seasonal nematode population fluctuations as statistical comparisons based on actual numbers of nematodes. The only significant drawback associated with the replacement series approach in studies involving pathogenic nematodes is that it does not include noninoculated controls. Such controls are required to document the effects of the pathogen on parameters such as plant weight, yield, or symptom expression.

The ecological relationship between root-knot and reniform nematode was initially defined on the root-knot susceptible soybean cultivar Davis in replacement series experiments conducted in a greenhouse. Although reniform nematodes extracted from soil outnumbered root-knot nematodes, populations of the latter were significantly larger when they developed on plants coinhabited by reniform nematode. This increase was not accompanied by suppression of the reniform population. The possibility existed that the increase in the root-knot population was merely a reflection of the migration of this species out of the root system. Thus, a rapid, efficient extraction procedure was designed to liberate vermiform and swollen individuals of both species from the root tissue. Grinding root tissue for 10 sec in 0.5% NaOCl was effective in liberating root-knot nematodes from soybean and tomato roots. Nematodes extracted using this procedure were used in conjunction with soil counts to calculate relative nematode yields in greenhouse and microplot experiments employing the soybean cultivar Davis. Results from the initial greenhouse tests were confirmed, as root-knot populations again were larger than expected in the presence of reniform nematode in

greenhouse tests. This indicated that the increase was due to enhanced reproduction rather than migration out of the root system. In microplot tests, the increase in the root-knot population likely occurred early in the season, which severely damaged the root system and made it less suitable for colonization by both species of nematodes. The ecological relationship between root-knot and reniform nematode as described herein was not detectable using standard ANOVA procedures based on actual nematode counts.

The mechanism(s) responsible for the increase in root-knot reproduction on Davis soybean in the presence of reniform nematode is unknown. Enhanced reproduction may be due to partial or complete loss of a low level of resistance, enhanced detection and penetration of roots by juvenile root-knot nematodes, or by physiological modification of the host to favor reproduction of root-knot nematode. Future research should be directed toward determination of the driving factors in this system.

The influence of additional biological factors on the relationship between root-knot and reniform nematode also was evaluated. Colonization of Davis soybean by the stem canker fungus did not alter the ecological relationship between the two nematodes. Investigations employing the root-knot resistant soybean cultivar Buckshot 66 revealed that the stimulatory effect of reniform nematode on root-knot nematode was absent in both greenhouse and microplot tests, suggesting that the inherent resistance of the host is the primary factor determining the nature of the relationship between these two nematode species.

Host suitability is another area that merits further study. Experiments to date have shown that reniform nematode has the ability to enhance root-knot nematode reproduction on soybean susceptible to the latter. The impact of higher root-knot

population densities on resistant and susceptible varieties of potential subsequent crops such as cotton should be investigated.

The research to date was conducted using an initial community density of 1,000 vermiform nematodes per greenhouse or microplot pot in tests lasting approximately 90 days. While this provides a point of reference for subsequent work, results from experiments of different durations, employing different community densities, or conducted under different soil, temperature, and moisture regimes are likely to yield vastly different results. The ecological and etiological relationships between pathogens can only be defined within the confines of the experimental system. However, the longest journey begins with the smallest step.

APPENDIX A

METHODOLOGY

Introduction

Establishment of several pathogens on a single host in the greenhouse is often tedious. Under these conditions, pathogens may not develop or may develop quite rapidly and kill the host in a short period of time. To study interrelationships between pathogens, methods allowing successful pathogen establishment and disease development over several months are essential. Studies can then be of sufficient duration to allow pathogens to interact with the host and each other, thus making it possible to monitor the effect of each pathogen on other components of the system.

Studies evaluating soybean growth stage at inoculation and isolate virulence of Diaporthe phaseolorum var. caulivora (Dpc) and inoculum sources and doses of Rhizoctonia solani (Rs) were conducted to improve the methods currently employed in greenhouse interrelationship studies.

Evaluation of Plant Growth Stage and Isolate Virulence of Diaporthe phaseolorum var. caulivora on Stem Canker Development

A greenhouse test was conducted to determine the relative virulence of three isolates of Dpc, causal agent of stem canker, on the susceptible soybean cultivar DeltaPine 105. Further, the effects of soybean growth stage at the time of inoculation on stem canker symptom development were examined.

Treatments consisted of two growth stages (V3, V6) and three isolates of the fungus (Ben Hur, Burden, Opelousas) in a factorial arrangement for a total of six treatment combinations. Each combination was replicated three times in a randomized complete block design.

At the appropriate growth stage, soybean stems were inoculated by inserting a toothpick section infested with one of the Dpc isolates into a small puncture made with a sterile needle between the unifoliate and first trifoliate nodes. Every 4 days, canker lengths were recorded and plant were observed for foliar symptoms.

Plants inoculated at V3 began showing stem canker leaf symptoms (red veins, curled leaves, wilted foliage) as soon as 6 days after inoculation (DAI) for all Dpc isolates tested. Additional foliar symptoms (chlorosis and necrosis of leaf tissue) were seen 10 DAI for all isolates. Cankers increased in length throughout the study period until plants were killed. Isolates could not be distinguished based on canker length (Figure A.1).

Plants inoculated at V6 began showing stem canker foliar symptoms 10 and 13 DAI for plants inoculated with the Ben Hur and Opelousas isolates, respectively. Plants inoculated with the Burden isolate did not exhibit leaf symptoms within the experimental period. Cankers increased in length throughout the study, and isolates could not be distinguished based on canker length (Figure A.2).

A comparison of plants inoculated at growth stages V3 and V6 revealed that canker lengths 10 DAI did not differ statistically (V3 = 48 mm, V6 = 27 mm, $\underline{F} = 2.46$, $\underline{P} > \underline{F} = 0.1475$). However, 13-14 DAI, cankers on plants inoculated at V3 (64 mm) were significantly longer than those on plants inoculated at V6 (34 mm) ($\underline{F} = 4.36$, $\underline{P} > \underline{F} = 0.0633$). This suggests that postponing the time of introduction of Dpc may result in slower disease development, thereby allowing more time for establishment of additional pathogens in disease complex studies.

Effect of Inoculum Sources and Levels on Colonization of Soybean by Rhizoctonia solani and on Aerial Blight Symptom Expression

A greenhouse test was conducted to determine which inoculation method(s) allow Rs AG-1 IA, causal agent of aerial blight, to colonize the soybean plant starting at the soil line, as it does under field conditions, and develop gradually.

Treatments consisted of three inoculum sources (5-mm-diam. plugs of Rs growing on potato dextrose agar, infested oat grains, 2- to 3-mm-diam. sclerotia) and two inoculum doses (1 per plant, 3 per plant) in a factorial arrangement for a total of

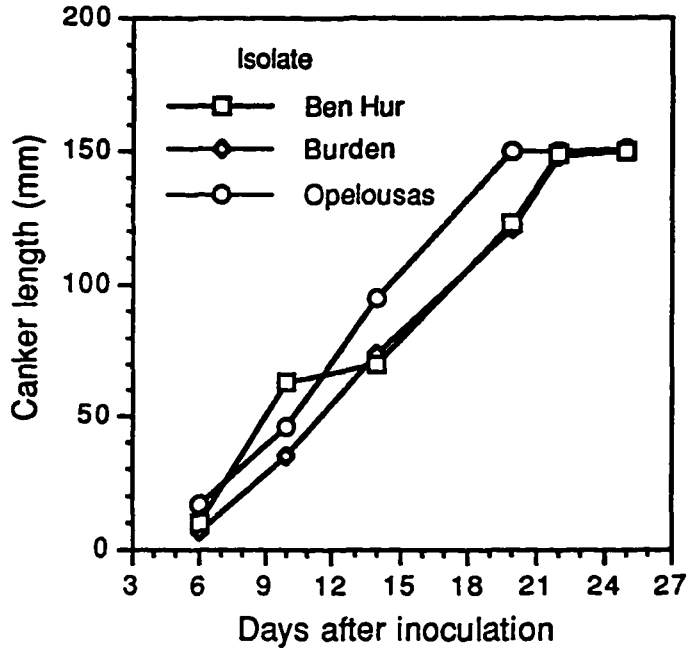

Fig. A.1. Canker lengths on DeltaPine 105 soybean plants inoculated at growth stage V3 with isolates of Diaporthe phaseolorum var. caulivora.

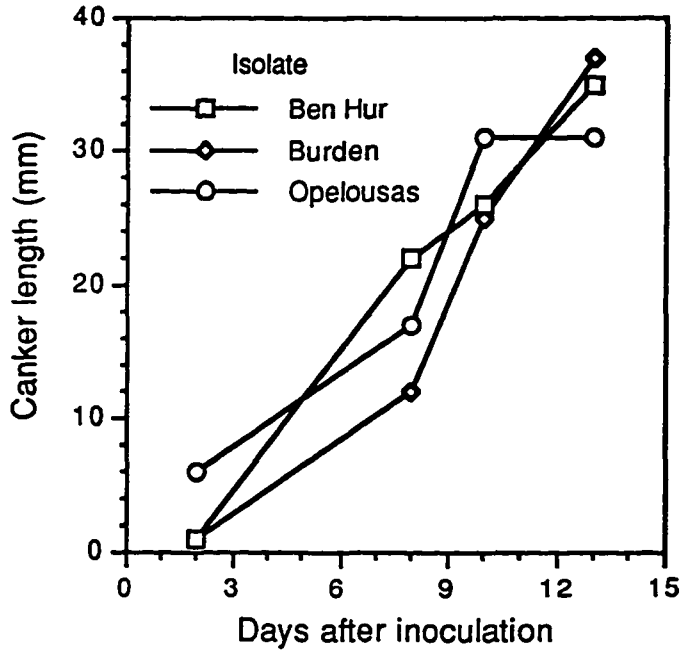

Fig. A.2. Canker lengths on DeltaPine 105 soybean plants inoculated at growth stage V6 with isolates of Diaporthe phaseolorum var. caulivora.

six treatment combinations. Each combination was replicated three times in a randomized complete block design.

Inoculum was placed just below the soil line at the base of DeltaPine 105 soybean plants at growth stage V3. The plants were placed in a plastic-covered chamber equipped with a cool-mist humidifier that ran continuously for 10 hours each night to keep a film of moisture on the plant surfaces. During the day, the chamber was opened and the plants were allowed to dry. Every 2-3 days, plants were examined for symptoms (water soaking, lesions) and signs (mycelium, sclerotia) of Rs. After 2 weeks, plants were harvested and two 3-mm sections of the main stem were taken every 50 mm up from the soil line. One section was surface sterilized (0.5% NaOCl, 2 min). Both sections were plated on 2% water agar and plates were examined to determine the presence/absence of Rs on and in plant tissues. Further, tissue from observed lesions was surface sterilized and plated to determine if Rs was associated with the lesions.

As soon as 2 DAI, mycelium was clearly visible for all inoculum sources except plugs; by 4 DAI, mycelium was observed on the surface of plants for all inoculum sources and doses. Tan lesions were observed beginning 4 DAI. Sclerotia were first noted 8 DAI and infected unifoliates were seen 10 DAI. Lesions, sclerotia, and infected leaves were seen for all inoculum sources and levels.

Rs was reisolated from all plants and was found colonizing the main stem approximately 50 mm further externally than internally. When plugs were used as the inoculum source, Rs was found farther up external stem surfaces than when grains were used. The distance travelled when sclerotia were the inoculum source was intermediate and indistinguishable from either plugs or grains. The mean internal distance colonized did not differ with respect to inoculum source (Figure A.3).

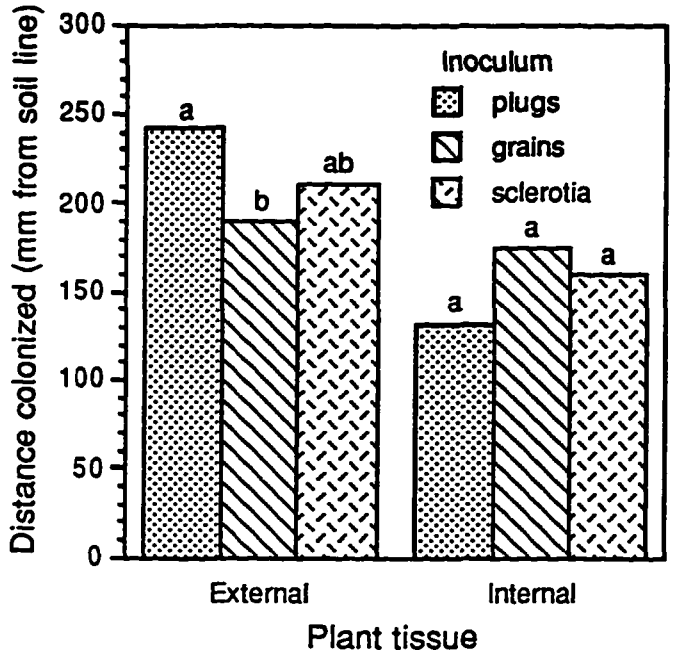

Fig. A.3. Soybean cv. DeltaPine 105 colonization by Rhizoctonia solani AG 1 IA from three inoculum sources; within each tissue, means with the same letter do not differ (Fisher's protected LSD, $P \leq 0.05$).

The mean distance from the soil line at which Rs was found either externally or internally was not affected by the level of inoculum used (Figure A.4).

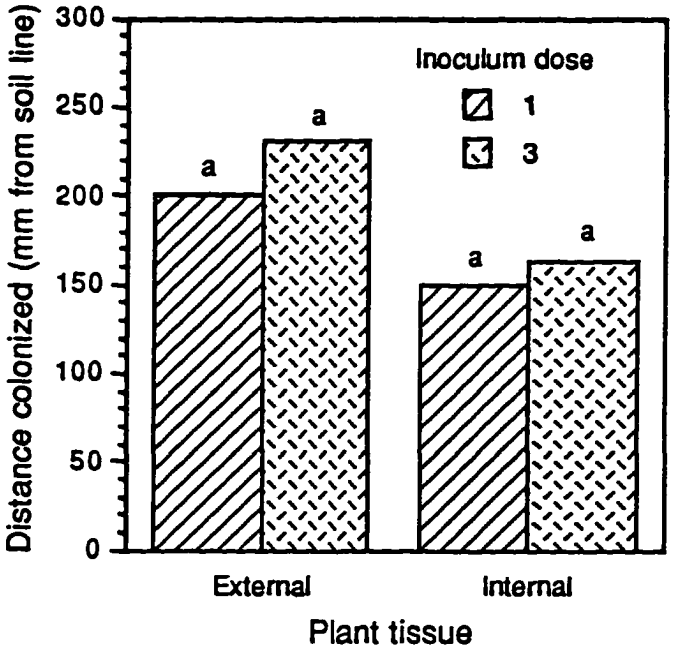

Fig. A.4. Soybean cv. DeltaPine 105 colonization by Rhizoctonia solani AG 1 IA at inoculum doses of one or three units of inoculum per plant; within each tissue, means with the same letter do not differ (Fisher's protected LSD, $P \leq 0.05$).

APPENDIX B

PEST COMPLEX RESEARCH

Introduction

Diseases of soybean rarely occur as individual entities. Instead, groups of interacting organisms are the rule in field situations. Two fungi that cause economic losses in Louisiana are Diaporthe phaseolorum var. caulivora (Dpc; stem canker) and Rhizoctonia solani (Rs; Rhizoctonia foliar blight). Two nematodes that commonly occur on soybean in Louisiana are Meloidogyne incognita (Mi; root-knot nematode) and Rotylenchulus reniformis (Rr; reniform nematode). Studies to identify the effects that these pathogens have on each other and on the soybean host were conducted from 1991-1994.

Greenhouse Evaluation of Interrelationships between Meloidogyne incognita and Rhizoctonia solani

The interrelationships between Mi and aerial blight or web blight isolates of Rs were evaluated on the soybean cultivars Riverside 757 (R 757; susceptible to Rs, resistant to Mi) and Hartz 8112 (H 8112; resistant to Rs, susceptible to Mi). Nematode treatments were soil not infested or infested with 400 juveniles per pot at V5. Fungus treatments were a noninoculated control, inoculation with Rs AG-1 IA (aerial blight; BHIA-10 isolate), and inoculation with Rs AG-1 IB (web blight; BHMS-1 isolate). Each leaflet of the second and fifth trifoliates down from the apex of each plant was inoculated with a 3-mm-diam. plug of mycelium growing on acidified potato dextrose agar (APDA) when plants were at growth stage R2; plugs of APDA without the fungus served as controls. Plants were misted for 96 hours, after which aerial blight or web blight severity was recorded. This experiment had a total of 12 treatment combinations (2 cultivar x 2 nematode x 3 fungus), each replicated five times in a randomized complete block design. Parameters measured at harvest were dry weight of foliage and roots, root gall rating, numbers of nematodes associated with host roots and soil, and aerial blight or web blight severity.

Foliage and root dry weights were not affected by nematode or fungus colonization of either cultivar (Table B.1). When the soil was infested with root-knot nematodes, less galling occurred on R 757 than on H 8112. Galling on either cultivar was not affected by Rs (Table B.2). Nematode reproduction was supported on these soybean cultivars, but the relatively low population levels at harvest (Table B.2) explain why no effects on plant weight parameters were detected. Aerial blight and web blight severities were equivalent on R 757, but aerial blight was more severe than web blight on H 8112 (Table B.3). Severity of aerial blight or web blight was not affected by nematode colonization on either cultivar. Only 20 days elapsed between inoculation with Rs and harvest. This short colonization period, in combination with limited growth of the isolates, explain why Rs did not affect plant weight parameters.

Microplot Evaluation of Interrelationships between *Meloidogyne incognita* and *Rhizoctonia solani*

The interrelationships between Mi and aerial blight or web blight isolates of Rs were evaluated on the soybean cultivars R 757 (susceptible to Rs, resistant to Mi) and H 8112 (resistant to Rs, susceptible to Mi). Nematode treatments were soil not infested or infested with 2,700 juveniles per pot at V6. Fungus treatments were a noninoculated control, inoculation with Rs AG-1 IA (aerial blight; BHIA-10 isolate), and inoculation with Rs AG-1 IB (web blight; BHMS-1 isolate). Microplot inoculation attempts using fungus-infested oats and agar plugs were not successful, so leaves detached when plants were at growth stage R5 were inoculated with plugs of Rs grown on APDA (1 plug per leaflet) and disease severity after 36 hours was recorded. This experiment had a total of 12 treatment combinations (2 cultivar x 2 nematode x 3 fungus), each replicated six times in a randomized complete block design. Parameters measured were aerial blight or web blight severity, number of pods and seeds; dry weight of seeds, foliage, and roots; gall rating, and number of nematodes in soil and roots. Since the

Table B.1. Mean foliage and root dry weight for Riverside 757 (R 757) and Hartz 8112 (H 8112) soybean at harvest.

Treatment	Level	Foliage weight (g)		Root weight (g)	
		R 757	H 8112	R 757	H 8112
Rs	absent	5.14	3.11	2.91	1.95
	BHIA-10	3.55	2.54	1.87	1.78
	BHMS-1	4.03	2.77	2.34	2.42
Mi	absent	4.22	2.80	2.69	2.02
	present	4.27	2.80	2.06	2.08
Source:					
Rs		NS	NS	NS	NS
Mi		NS	NS	NS	NS

Within each cultivar, NS = means are not significantly different at $P \leq 0.05$.

Table B.2. Influence of Rhizoctonia solani on galling and on nematode populations in soil (number/pot) and roots (eggs/root system) on Riverside 757 (R 757) and Hartz 8112 (H 8112) soybean at harvest.

Rhizoctonia solani	Galling[a]		Soil		Roots	
	R 757	H 8112	R 757	H 8112	R 757	H 8112
absent	1	3	2,710	9,420	204	712
BHIA-10	1	3	4,358	9,446	255	703
BHMS-1	1	2	4,582	9,653	254	695
Source:						
Rs	NS	NS	NS	NS	NS	NS

Within each parameter and cultivar, NS = means are not significantly different at $P \leq 0.05$.

[a] Rating is a product of severity and incidence values described in Chapter 2 (0 to 20 scale where 0=no galls and 20=entire root system severely galled).

Table B.3. Mean aerial blight or web blight severity (% of leaf affected) for Riverside 757 (R 757) and Hartz 8112 (H 8112) soybean 96 hours after inoculation with Rhizoctonia solani.

Treatment	Level	Aerial blight/web blight severity	
		R 757	H 8112
Rs	absent	0.0 a	0.0 a
	BHIA-10	1.7 b	2.1 b
	BHMS-1	0.6 ab	0.0 a
Mi	absent	0.6 a	1.0 a
	present	0.9 a	0.4 a

Within each cultivar and treatment, means followed by the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$). Significance was determined after square root transformation of severity data.

fungus was not established in the microplot, it had no opportunity to affect the number of pods and seeds, dry weight, nematode numbers, or galling induced by nematodes.

Aerial blight and web blight severity was not affected by nematode colonization on either cultivar (Table B.4). Nematode colonization of root systems did not affect the number of seeds produced by R 757, but significantly reduced the number of seeds produced by H 8112 (Table B.5). Nematode colonization did not affect the number of pods produced by either cultivar (Table B.5). Root, foliage, and seed dry weights for R 757 and H 8112 were not significantly affected by nematode colonization (Table B.6). Root and soil Mi populations did not differ between cultivars, though galling on H 8112 was worse than that observed on R 757 (Table B.7).

Interrelationships between *Rhizoctonia solani*, *Meloidogyne incognita*, and *Rotylenchulus reniformis*

The relationship between Rs AG-1 IA (aerial blight) and nematodes (Mi or Rr) was examined on soybean in greenhouse tests employing the cultivars R 757 (susceptible to Rs and Rr, resistant to Mi) and H 8112 (resistant to Rs, susceptible to Rr and Mi). Treatments were fungus (inoculated, noninoculated) and nematode (no nematodes, Mi, Rr), in a factorial arrangement for a total of six treatment combinations. There were three replicates of each treatment combination including Mi; all other treatment combinations were replicated five times. Soybean leaves were inoculated with Rs by pressing a 3-mm-diam. plug of the fungus (from a culture grown on APDA) onto the center of the upper surface of each leaflet of 3-4 trifoliates on each plant. Noninoculated treatments received plugs of APDA. The greenhouse bench was then covered with clear plastic, and vaporizers were used to keep the relative humidity at or above 95%, to create a favorable environment for growth of the fungus. Trifoliates were inoculated 3, 5, and 8 weeks after nematodes were introduced into the pots (plants at growth stages V11, R1, and R2, respectively). The soil in each pot was infested with

Table B.4. Effect of inoculation with Meloidogyne incognita on severity (% of leaf affected) of aerial blight and web blight on Riverside 757 and Hartz 8112 soybean in detached leaf assays.

Cultivar	Nematode	Disease severity	
		Aerial blight	Web blight
Riverside 757	absent	13.4 a	11.2 a
	present	13.5 a	18.0 a
Hartz 8112	absent	12.4 a	9.4 a
	present	11.7 a	15.9 a

Within each cultivar and disease, means followed by the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$).

Table B.5. Influence of Meloidogyne incognita on seed and pod production (number/plant) on Riverside 757 (R 757) and Hartz 8112 (H 8112) soybean at harvest.

	Seeds/plant		Pods/plant	
Nematode	R 757	H 8112	R 757	H 8112
absent	73 a	32 a	36 a	17 a
present	50 a	14 b	25 a	10 a

Within each parameter and cultivar, means followed by the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$).

Table B.6. Influence of Meloidogyne incognita on root, foliage, and seed dry weight (g/plant) for Riverside 757 (R 757) and Hartz 8112 (H 8112) soybean at harvest.

Nematode	Root		Foliage		Seed	
	R 757	H 8112	R 757	H 8112	R 757	H 8112
absent	2.3 a	1.3 a	9.8 a	4.3 a	2.9 a	1.0 a
present	2.5 a	1.1 a	5.5 a	3.3 a	1.7 a	0.4 a

Within each parameter and cultivar, means followed by the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$).

Table B.7. Influence of soybean cultivar on galling and on Meloidogyne incognita populations in soil (number/pot) and roots (eggs/root system) at harvest.

Cultivar	Galling[a]	Number of nematodes	
		Soil	Roots
Riverside 757	1 b	41 a	17,151 a
Hartz 8112	6 a	49 a	13,610 a

Within each parameter, means followed by the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$).

[a] Rating is a product of severity and incidence values described in Chapter 2 (0 to 20 scale where 0=no galls and 20=entire root system severely galled).

400 nematodes (mixed vermiform life stages) when the plants reached growth stage V6. Parameters measured were aerial blight severity, foliage and root dry weights, gall ratings, number of nematodes from soil and host roots, and nutrient composition of soybean leaves.

Aerial blight severity on Rs-infected plants was not significantly affected by nematode colonization of the root system 3 and 5 wk after infestation with nematodes for R 757 and H 8112 (Table B.8). Further, no significant nematode effects were detected 8 wk after nematode infestation on R 757 (Table B.8). However, there were significant fungus and nematode main effects and a significant fungus x nematode interaction detected on H 8112 8 wk after nematode infestation (Table B.9). It appears that an additive relationship exists between Mi and Rs with respect to aerial blight severity on H 8112.

Neither fungus nor nematode treatments affected foliage dry weight on either cultivar (Table B.10). Root dry weight was not affected by either the fungus or nematodes on H 8112 (Table B.10). On R 757, root dry weight was not affected by Rs, but heavier roots were recovered from plants parasitized by Rr than from control or Mi-parasitized plants (Table B.10).

Mean numbers of nematodes associated with soil or roots were not significantly affected by Rs on R 757 (Table B.11) or H 8112 (Table B.12). On R 757, galling induced by Mi was not affected by Rs (Table B.11). However, galling was worse on H 8112 infected with Rs than on fungus-free plants (Table B.12).

On both cultivars, several nutrients in soybean leaves were correlated with nematode population sizes and aerial blight severity (Table B.13).

Table B.8. Aerial blight severity (% of leaf affected) on Riverside 757 (R 757) and Hartz 8112 (H 8112) soybean not colonized or colonized by Meloidogyne incognita (Mi) or Rotylenchulus reniformis (Rr).

Nematode	Weeks after nematode infestation				
	3		5		8
	R 757	H 8112	R 757	H 8112	R 757
none	61 a	59 a	8 a	31 a	29 a
Mi	59 a	64 a	11 a	10 a	34 a
Rr	63 a	61 a	8 a	31 a	8 a

Within week and cultivar, means followed by the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$).

Table B.9. Influence of inoculation with Rhizoctonia solani on aerial blight severity (% of leaf affected) on Hartz 8812 soybean not infested with nematodes or 8 wk after infestation with Meloidogyne incognita, or Rotylenchulus reniformis.

Treatment	Level	Aerial blight severity
Fungus	none	0 b
	Rhizoctonia solani	13 a
Nematode	none	2 b
	Meloidogyne incognita	14 a
	Rotylenchulus reniformis	2 b
Fungus x nematode	neither	0 b
	M. incognita	0 b
	R. reniformis	0 b
	R. solani	5 b
	R. solani and M. incognita	28 a
	R. solani and R. reniformis	5 b

Within each treatment, means followed by the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$).

Table B.10. Influence of Rhizoctonia solani, Meloidogyne incognita, and Rotylenchulus reniformis on foliage and root dry weight (g/plant) at harvest of Riverside 757 (R 757) and Hartz 8112 (H 8112) soybean.

		Foliage weight		Root weight	
Treatment	Level	R 757	H 8112	R 757	H 8112
Fungus	none	12 a	16 a	6 a	13 a
	R. solani	11 a	15 a	6 a	11 a
Nematode	none	11 a	16 a	6 b	13 a
	M. incognita	12 a	14 a	3 b	9 a
	R. reniformis	11 a	16 a	9 a	14 a

Within each treatment and parameter, means followed by the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$).

Table B.11. Influence of Rhizoctonia solani on populations of Rotylenchulus reniformis (Rr) and Meloidogyne incognita (Mi) associated with soil (number/pot) and roots (eggs/root system), and on galling induced by Mi on Riverside 757 soybean.

	Rotylenchulus reniformis		Meloidogyne incognita		
Fungus	Soil	Roots	Soil	Roots	Galling[a]
none	3,644 a	112 a	2,050 a	273 a	1 a
R. solani	3,248 a	77 a	4,700 a	309 a	1 a

Within each parameter, means followed by the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$).

[a] Rating is a product of severity and incidence values described in Chapter 2 (0 to 20 scale where 0=no galls and 20=entire root system severely galled).

Table B.12. Influence of Rhizoctonia solani on populations of Rotylenchulus reniformis (Rr) and Meloidogyne incognita (Mi) associated with soil (number/pot) and roots (eggs/root system), and on galling induced by Mi on Hartz 8112 soybean.

	Rotylenchulus reniformis		Meloidogyne incognita		
Fungus	Soil	Roots	Soil	Roots	Galling[a]
none	6,778 a	322 a	10,533 a	916 a	3 b
R. solani	7,758 a	268 a	10,883 a	933 a	5 a

Within each parameter, means followed by the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$).

[a] Rating is a product of severity and incidence values described in Chapter 2 (0 to 20 scale where 0=no galls and 20=entire root system severely galled).

Table B.13. Correlations between nematode population size or aerial blight severity and foliar nutrient concentration on Riverside 757 (R 757) and Hartz 8112 (H 8112) soybean.

Cultivar	Parameter	Nutrient	Correlation coefficient	$P > \|r\|$
R 757	root population	Mn	+ 0.40140	0.0421
	soil population	Zn	+ 0.54135	0.0052
	aerial blight severity	Al	+ 0.48570	0.0138
H 8112	root population	Ni	+ 0.43314	0.0345
		Al	- 0.42575	0.0381
	soil population	Cu	+ 0.59076	0.0024
		Ni	+ 0.70779	0.0001
		Co	+ 0.48069	0.0174
	aerial blight severity	Mg	+ 0.41506	0.0437

Interrelationships between *Rhizoctonia solani*, *Diaporthe phaseolorum* var. *caulivora*, and *Rotylenchulus reniformis* on Sharkey Soybean

A greenhouse experiment was conducted to evaluate the interrelationships between Rs AG-1 IA, Dpc, and Rr on Sharkey soybean. Treatments were Rs (present, absent), Dpc (present, absent), and Rr (0/pot, 500/pot, 1,000/pot) in a factorial arrangement. Each treatment combination was replicated five times in a randomized block design.

Soil was infested with nematodes when the plants were at V3. Six weeks later, plants at R2 were inoculated with Dpc (Burden isolate) by inserting an infested toothpick sections into a puncture in the stem between the unifoliate and first trifoliate nodes. Sterile toothpick sections were inserted into control plants. Four weeks after the introduction of Dpc, plants were inoculated with a suspension of mycelial fragments of 3-day-old potato dextrose agar (PDA) cultures of Rs ground in distilled water (10.5×10^6 fragments/plant). PDA ground in distilled water was sprayed onto control plants. Following inoculation with Rs, plants were placed under a mist system and kept moist for 72 hours, after which plants were misted only at night. Stem canker lesion length was recorded at 10-day intervals following inoculation with Dpc. At harvest, (13 weeks after nematode infestation), parameters measured were aerial blight severity, foliage and root dry weights, and numbers of nematodes in soil and roots.

Dpc, Rs, and Rr did not affect the dry weight of plant roots or foliage (Table B.14). Canker lengths on Dpc-infected plants were not affected by either Rs or Rr 8, 19, and 30 days after inoculation (DAI) with Dpc. At 44 DAI, cankers were shorter on plants infected with Rs, though their length was not affected by Rr (Table B.15). Rs had colonized these plants for 4 weeks. Dpc and Rr did not affect severity of aerial blight severity on Rs-infected plants (Table B.16). Nematode population data at harvest

Table B.14. Influence of Diaporthe phaseolorum var. caulivora (Dpc), Rhizoctonia solani (Rs), and Rotylenchulus reniformis (Rr) on dry weights (g/plant after 96 hours at 70°C) of roots and foliage at harvest for Sharkey soybean.

Treatment	Level	Root weight	Foliage weight
Dpc	present	12.7	26.7
	absent	10.6	25.9
Rs	present	12.6	27.2
	absent	10.6	25.4
Rr	0/pot	12.7	28.2
	500/pot	11.6	25.6
	1,000/pot	10.7	25.1
Source:			
Dpc		NS	NS
Rs		NS	NS
Rr		NS	NS

NS = means are not significantly different ($P \leq 0.05$).

Table B.15. Influence of Rhizoctonia solani (Rs), and Rotylenchulus reniformis (Rr) on canker lengths (mm) on Sharkey soybean 8, 19, 30, and 44 days after inoculation (DAI) with Diaporthe phaseolorum var. caulivora.

		Canker length			
Treatment	Level	8 DAI	19 DAI	30 DAI	44 DAI
Rs	present	4	9	21	47
	absent	4	11	25	118
Rr	0/pot	3	9	22	82
	500/pot	5	12	21	78
	1,000/pot	4	8	26	87
Source:					
Rs		NS	NS	NS	**
Rr		NS	NS	NS	NS

** Means are significantly different at $P \leq 0.01$ based on the F test; NS = means are not significantly different ($P \leq 0.05$).

Table B.16. Influence of Diaporthe phaseolorum var. caulivora (Dpc) and Rotylenchulus reniformis (Rr) on aerial blight severity (% of plant organ diseased) at harvest for Sharkey soybean infected with Rhizoctonia solani.

		Aerial blight severity			
Treatment	Level	Stems	Leaves	Pods	Average
Dpc	present	21	34	21	25
	absent	30	41	27	33
Rr	0/pot	19	28	19	22
	500/pot	25	37	28	30
	1,000/pot	33	48	26	36
Source:					
Dpc		NS	NS	NS	NS
Rr		NS	NS	NS	NS

NS = means are not significantly different ($P \leq 0.05$). Significance was determined after arc sin transformation of severity data.

(90 days after nematode infestation) are summarized in Table B.17. Values for Pf/Pi, a measure of nematode reproduction, were not affected by Dpc, Rs, or Rr. These values were all less than 1.0, indicating that Sharkey does not support Rr reproduction. Neither Dpc nor Rs affected root or soil nematode populations. Numbers of nematodes associated with host roots did not differ with respect to the density of nematodes at the time of soil infestation. Numbers of coiled juveniles, active juveniles, and total nematodes in the soil increased as the density of nematodes at the time of soil infestation increased; the relationship was linear.

Interrelationships between Diaporthe phaseolorum var. caulivora, Rhizoctonia solani, and Rotylenchulus reniformis on Davis Soybean

A greenhouse experiment was conducted to evaluate the interrelationships between Dpc (Burden isolate), Rs AG-1 IA (BHIA-10 isolate), and Rr on Davis soybean. Treatments were Dpc (present, absent), Rs (present, absent), and Rr (0, 500, or 1,000 vermiform nematodes/pot) in a factorial arrangement. Each treatment combination was replicated five times in a randomized complete block design.

Soil in each pot was infested with nematodes one week after a 10-day-old seedling was transplanted into the pot. Six weeks later, plants were inoculated by inserting Dpc-infested toothpick sections into a puncture made between the unifoliate and first trifoliate nodes; sterile toothpick sections were inserted into control plants. Three weeks after the introduction of Dpc, plants were inoculated with a suspension of mycelial fragments of 2-day-old PDA cultures of Rs ground in distilled water (2×10^6 fragments/plant). A suspension of PDA ground in distilled water was sprayed onto control plants. Following inoculation with Rs, plants were placed under a mist system and kept moist for 72 hours, after which plants were misted only at night. Stem canker lesion length was recorded at 10-day intervals following inoculation with Dpc. At harvest,

Table B.17. Influence of Rotylenchulus reniformis (Rr) inoculum level, Rhizoctonia solani (Rs), and Diaporthe phaseolorum var. caulivora (Dpc) on populations of Rr on Sharkey soybean 90 days after nematode infestation.

Treatment	Level	Rr stages in soil[a]					Pf/Pi[b]	Rr stages in roots	
		Coiled juveniles	Active juveniles	Males	Females	Total		Sessile females[c]	Eggs/root system
Dpc	present	42	15	9	6	72	0.15	1	0
	absent	29	20	14	11	77	0.13	0	0
Rs	present	38	23	20	12	94	0.18	1	0
	absent	34	12	3	6	55	0.09	0	0
Rr	0	0	0	0	0	0	---	0	0
	500	30	9	8	4	52	0.10	0	0
	1,000	78	43	26	21	172	0.17	2	0
Contrast:	Linear	***	**	---	---	***	---	---	---
	Quadratic	NS	NS	---	---	NS	---	---	---
Source:									
Dpc		NS	NS	NS	NS	NS	NS	NS	NS
Rs		NS	NS	NS	NS	NS	NS	NS	NS
Rr		***	*	NS	NS	***	NS	NS	NS

[a] Nematodes per 15-cm-diam. pot (1.6 kg soil).

[b] Pi = initial Rr infestation level, Pf = final Rr population density in soil.

[c] Females per 10 2.5-cm root pieces.

*, **, *** = means are significantly different at $P \leq 0.05$, 0.01, and 0.001, respectively; NS = means are not significantly different. Significance was determined after log transformation of nematode numbers from soil and eggs/root system and after square root transformation of numbers of sessile females.

parameters measured were aerial blight severity, plant dry weight, number and area of nonnecrotic leaflets, and numbers of nematodes in soil and roots.

Canker lengths 10 and 20 days after inoculation (DAI) with Dpc were not affected by colonization of plants by either Rs or Rr. However at 30 DAI, cankers were shorter on plants colonized by Rr (Table B.18). The length of time during which Dpc and Rs were coexisting on the plant may not have been sufficient to detect any impact of Rs on the relatively slow development of cankers induced by Dpc. A significant Dpc x Rr interaction was detected with regard to aerial blight severity 14 DAI with Rs (Table B.19). Severities were significantly lower on plants colonized by Dpc for those treatments with initial nematode infestation levels of 0 or 1,000/pot (Figure B.1). Reduced aerial blight severity is probably an indirect result of a Dpc- or Rr-induced alteration in the physiological state of the host plant. Dpc-colonized plants had significantly fewer leaflets and smaller nonnecrotic leaf areas at harvest than respective controls (Table B.20). Leaflet number and area were not affected by colonization with Rs or Rr. The reduction in leaf area associated with colonization of plants by Dpc did not translate into a reduction in plant dry weight, as none of the pathogens affected plant weight (Table B.21). Nematode populations associated with soil and roots were not influenced by colonization of the soybean by either Dpc or Rs. The Pf/Pi ratio was not influenced by colonization of the host by either fungus (Table B.22).

Additional Investigations into the Relationship between *Rhizoctonia solani*, *Diaporthe phaseolorum* var. *caulivora*, and *Rotylenchulus reniformis* on Davis Soybean

A greenhouse experiment was conducted to evaluate the interrelationships between Rs AG-1 IA, Dpc, and Rr on Davis soybean. Treatments were Rs (present, absent), Dpc (present, absent), and Rr (0/pot, 500/pot, 1,000/pot) in a factorial arrangement. Each treatment combination was replicated five times in a randomized block design.

Table B.18. Influence of Rhizoctonia solani (Rs) and Rotylenchulus reniformis (Rr) on stem canker lengths (mm) on Davis soybean 10, 20, and 30 days after inoculation (DAI) with Diaporthe phaseolorum var. caulivora.

		Canker length		
Treatment	Level	10 DAI	20 DAI	30 DAI
Rs	present	5.5	29.3	62.5
	absent	3.9	35.1	55.2
Rr	0	5.8	44.8	81.6 a
	500	3.4	28.2	48.1 b
	1,000	4.9	23.6	47.0 b
Source:				
	Rs	NS	NS	NS
	Rr	NS	NS	*

* Means are significantly different at $P \leq 0.05$; NS = means are not significantly different.

Means separation based on Fisher's protected LSD ($P \leq 0.05$).

Table B.19. Influence of Diaporthe phaseolorum var. caulivora (Dpc) and Rotylenchulus reniformis (Rr) on aerial blight severity (% of leaves diseased) on Davis soybean 14 days after inoculation with Rhizoctonia solani.

Treatment	Level	Aerial Blight Severity[a]
Dpc	present	4.4
	absent	13.9
Rr	0	6.9
	500	8.3
	1,000	12.3
Source:		
Dpc		***
Rr		NS
Dpc x Rr		*

*, *** Means are significantly different at $P \leq 0.05$ and 0.001, respectively; NS = means are not significantly different.

[a] Significance determined after arc sine transformation of data.

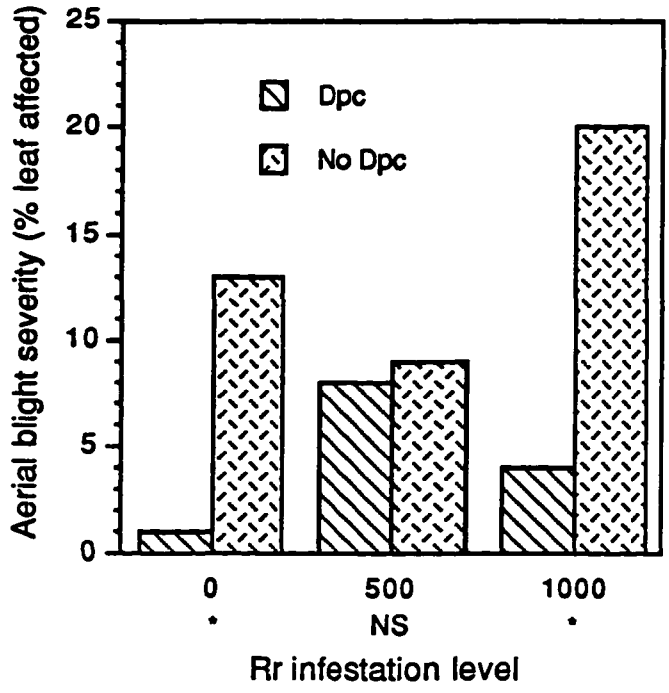

Fig. B.1. Effects of Diaporthe phaseolorum var. caulivora (Dpc) and Rotylenchulus reniformis (Rr) on severity of aerial blight on Davis soybean. * = means are significantly different at $P \leq 0.05$; NS = means are not significantly different.

Table B.20. Effects of Diaporthe phaseolorum var. caulivora (Dpc), Rhizoctonia solani (Rs), and Rotylenchulus reniformis (Rr) on the number and area of nonnecrotic leaflets of Davis soybean at harvest.

Treatment	Level	Number of leaflets	Leaflet area (cm^2)
Dpc	present	13	174
	absent	36	428
Rs	present	26	341
	absent	24	261
Rr	0	17	205
	500	30	377
	1,000	27	321
Source:			
Dpc		***	**
Rs		NS	NS
Rr		NS	NS

*, *** Means are significantly different at $P \leq 0.05$ and 0.001, respectively; NS = means are not significantly different.

Table B.21. Effects of Diaporthe phaseolorum var. caulivora (Dpc), Rhizoctonia solani (Rs), and Rotylenchulus reniformis (Rr) on Davis soybean dry weight (g/plant after 96 hours at 70°C).

		Dry weight		
Treatment	Level	Root	Shoot	Total
Dpc	present	1.37	1.87	3.24
	absent	1.43	2.51	3.94
Rs	present	1.54	2.34	3.88
	absent	1.26	2.04	3.30
Rr	0	1.33	2.08	3.41
	500	1.42	2.34	3.75
	1,000	1.45	2.16	3.61
Source:				
Dpc		NS	NS	NS
Rs		NS	NS	NS
Rr		NS	NS	NS

NS = means are not significantly different ($P \leq 0.05$).

Table B.22. Effects of Rotylenchulus reniformis (Rr) inoculum level, Diaporthe phaseolorum var. caulivora (Dpc), and Rhizoctonia solani (Rs) on population densities of Rr on Davis soybean 80 days after infestation with nematodes.

Treatment	Level	Life Stages in Soil				Eggs/Root System	Pf/Pi[a]
		Juveniles	Males	Females	Total		
Dpc	present	766	57	13	836	359	2
	absent	886	97	10	993	521	2
Rs	present	808	84	16	907	351	2
	absent	843	70	7	921	529	2
Rr	0	0	0	0	0	0	0
	500	942	92	18	1,052	644	4
	1,000	1,536	139	18	1,692	675	2
Contrast:	Linear	***	***	*	***	***	***
	Quadratic	***	***	NS	***	***	***
Source:	Dpc	NS	NS	NS	NS	NS	NS
	Rs	NS	NS	NS	NS	NS	NS
	Rr	***	***	*	***	***	***

[a] Pi = initial Rr infestation level, Pf = final Rr population density in soil.
Significance was determined after log transformation of nematode numbers.
*, *** = means are significantly different at $P \leq 0.05$ and 0.001, respectively; NS = means are not significantly different.

Soil was infested with nematodes when the plants were at V3. Six weeks later, when plants were at R2, Dpc (Burden isolate) was introduced into the plants on infested toothpick sections inserted into a puncture in the stem between the unifoliate and first trifoliate nodes. Sterile toothpick sections were inserted into control plants. Two weeks after the introduction of Dpc, plants were inoculated with a suspension of mycelial fragments of 3-day-old PDA cultures of Rs ground in distilled water (10.5×10^6 fragments/plant). PDA ground in distilled water was sprayed onto control plants. Following inoculation with Rs, plants were placed under a mist system and kept moist for 72 hours, after which plants were misted only at night. Stem canker lesion length was recorded at 10-day intervals following inoculation with Dpc. At harvest (13 weeks after nematode infestation), parameters measured were aerial blight severity, foliage and root dry weights, leaf number and leaf area, pod numbers, seed numbers and dry weights, and numbers of nematodes in soil and roots. Viability of a subsample of eggs recovered from roots was also determined.

Canker lengths on Dpc-infected plants were not affected by either Rs or Rr at any time during the study period (Table B.23). Aerial blight severity at harvest on Rs-infected plants was not affected by either Dpc or Rr (Table B.24). Dpc reduced total leaf area per plant (Table B.25). This reduction is a result of fewer leaves per plant rather than less area per leaf (Table B.25). Dpc also reduced the total seed dry weight per plant (Table B.26). In this case, the reduction was due to fewer seeds as well as lighter seeds (Table B.26). Rs and Rr did not affect seed and leaf parameters. Plants infected with Dpc had fewer pods and a lower proportion of green pods than control plants (Table B.27). Rs and Rr did not affect these parameters. Dpc-infected plants had lower root and foliage dry weights than control plants (Table B.28). Further, the proportion of the foliage that was green was reduced in the presence of Dpc.

Table B.23. Influence of Rhizoctonia solani (Rs) and Rotylenchulus reniformis (Rr) on canker lengths (mm) on Davis soybean 19, 30, and 40 days after inoculation (DAI) with Diaporthe phaseolorum var. caulivora.

		Canker length		
Treatment	Level	19 DAI	30 DAI	40 DAI
Rs	present	11	26	45
	absent	7	41	63
Rr	0/pot	9	28	38
	500/pot	7	44	69
	1,000/pot	10	28	54
Source:				
Rs		NS	NS	NS
Rr		NS	NS	NS

NS = means are not significantly different ($P \leq 0.05$).

Table B.24. Influence of Diaporthe phaseolorum var. caulivora (Dpc) and Rotylenchulus reniformis (Rr) on aerial blight severity (% of plant organ diseased) at harvest on Davis soybean inoculated with Rhizoctonia solani.

		Aerial blight severity[a]			
Treatment	Level	Stems	Leaves	Pods	Average
Dpc	present	25	40	26	30
	absent	35	43	30	36
Rr	0/pot	23	35	24	27
	500/pot	30	45	33	36
	1,000/pot	37	45	28	37
Source:					
Dpc		NS	NS	NS	NS
Rr		NS	NS	NS	NS

NS = means are not significantly different ($P \leq 0.05$).

[a] Significance was determined after arc sin transformation of severity data.

Table B.25. Effect of Diaporthe phaseolorum var. caulivora (Dpc), Rhizoctonia solani (Rs), and Rotylenchulus reniformis (Rr) on mean number and area of green leaves on Davis soybean at harvest.

Treatment	Level	Leaf area (cm^2)	Leaves/plant	Area/leaf (cm^2)
Dpc	present	425.4	15	28.2
	absent	695.2	28	24.8
Rs	present	559.9	21	25.8
	absent	560.7	22	27.2
Rr	0/pot	501.3	18	25.9
	500/pot	498.0	19	28.7
	1,000/pot	681.7	27	24.8
Source:				
Dpc		*	**	NS
Rs		NS	NS	NS
Rr		NS	NS	NS

*, ** = means are significantly different at $P \leq 0.05$ and 0.01, respectively; NS = means are not significantly different.

Table B.26. Effect of Diaporthe phaseolorum var. caulivora (Dpc), Rhizoctonia solani (Rs), and Rotylenchulus reniformis (Rr) on number and dry weight of green seeds for Davis soybean at harvest.

Treatment	Level	Seed dry weight (g)	Seeds/plant	Dry weight/seed (g)
Dpc	present	1.85	34	0.04
	absent	3.62	51	0.07
Rs	present	2.54	41	0.07
	absent	2.92	43	0.05
Rr	0/pot	2.89	46	0.06
	500/pot	2.57	39	0.05
	1,000/pot	2.73	42	0.06
Source:				
Dpc		***	**	**
Rs		NS	NS	NS
Rr		NS	NS	NS

, * = means are significantly different at $P \leq 0.01$, and 0.0001, respectively; NS = means are not significantly different.

Table B.27. Effect of Diaporthe phaseolorum var. caulivora (Dpc), Rhizoctonia solani (Rs), and Rotylenchulus reniformis (Rr) on pod production of Davis soybean at harvest.

Treatment	Level	Pods/plant	% Green pods
Dpc	present	29	39
	absent	37	66
Rs	present	32	56
	absent	33	49
Rr	0/pot	33	52
	500/pot	32	49
	1,000/pot	34	58
Source:			
Dpc		*	**
Rs		NS	NS
Rr		NS	NS

*, ** = means are significantly different at $P \leq 0.05$ and 0.01, respectively; NS = means are not significantly different.

Table B.28. Effect of Diaporthe phaseolorum var. caulivora (Dpc), Rhizoctonia solani (Rs), and Rotylenchulus reniformis (Rr) on Davis soybean root and foliage dry weight (g/plant after 96 hours at 70°C) at harvest.

Treatment	Level	Root weight	Foliage weight	% Green foliage
Dpc	present	4.88	11.25	47
	absent	6.89	13.89	83
Rs	present	6.81	13.18	71
	absent	4.97	11.95	59
Rr	0/pot	5.30	13.03	62
	500/pot	6.13	11.79	57
	1,000/pot	6.23	12.87	75
Source:				
Dpc		*	*	***
Rs		*	NS	NS
Rr		NS	NS	NS

*, *** = means are significantly different at $P \leq 0.05$ and 0.0001, respectively; NS = means are not significantly different.

Rs-infected plants had larger root dry weights than respective control plants. Rr did not influence plant weight (Table B.28).

Nematode population data at harvest (90 days after nematode infestation) are summarized in Table B.29. Values for Pf/Pi, a measure of nematode reproduction, were greater at lower initial infestation levels of Rr than at higher levels. Pf/Pi values were not affected by either Rs or Dpc. The high Pf/Pi values indicated that Davis is a suitable host for Rr. Soil life stages were not affected by Dpc or Rs. As Rr density at infestation increased, the numbers of soil nematodes of all life stages increased. The relationship was linear for all life stages except females; in this case, the relationship was quadratic. As initial Rr density increased, the number of sessile females increased; the relationship was linear. Dpc and Rs did not affect numbers of sessile females. The number of eggs per root system was reduced in the presence of Dpc, but was not affected by Rs. As initial Rr density increased, eggs per root system increased; the relationship was linear.

An interaction was detected between Dpc and Rr regarding the number of eggs per root system (Table B.29). At the low initial Rr density, there were fewer eggs on Dpc-infected plants than on noninoculated controls. At the high initial Rr density, Dpc did not affect the number of eggs per root system (Table B.30).

Egg hatch was not affected by Dpc or Rs alone, but a Dpc x Rs interaction was detected at the high Rr infestation level (Table B.31). At the high initial Rr level, Dpc increased egg hatch only on plants not infected with Rs, and Rs increased egg hatch only on plants not infected with Dpc (Table B.32).

Table B.29. Influence of Rotylenchulus reniformis (Rr) inoculum level, Rhizoctonia solani (Rs), and Diaporthe phaseolorum var. caulivora (Dpc) on populations of Rr on Davis soybean 90 days after nematode infestation.

Treatment	Level	Rr stages in soil[a]					Pf/Pi[b]	Rr stages in roots	
		Coiled juveniles	Active juveniles	Males	Females	Total		Sessile females[c]	Eggs/root system
Dpc	present	28,128	65,800	6,798	287	101,013	208.4	1	6,905
	absent	18,767	57,598	5,039	177	81,580	191.1	1	11,793
Rs	present	26,702	71,868	5,966	133	104,668	231.1	1	10,989
	absent	20,193	51,530	5,871	330	77,925	168.4	2	7,708
Rr	0	0	0	0	0	0	---	0	0
	500	32,133	84,768	8,256	463	125,620	251.2	2	12,396
	1,000	38,209	100,329	9,500	232	148,270	148.3	3	15,650
Contrast:	Linear	***	***	***	NS	***	---	***	***
	Quadratic	***	***	***	***	***	---	NS	***
Source:									
Dpc		NS	NS	NS	NS	NS	NS	NS	*
Rs		NS	NS	NS	NS	NS	NS	NS	NS
Rr		***	***	***	**	***	*	**	***
Dpc x Rs		NS	NS	NS	NS	NS	NS	NS	NS
Dpc x Rr		NS	NS	NS	NS	NS	NS	NS	*
Rs x Rr		NS	NS	NS	NS	NS	NS	NS	NS
Dpc x Rs x Rr		NS	NS	NS	NS	NS	NS	NS	NS

[a] Nematodes per 15-cm-diam. pot (1.6 kg soil).

[b] Pi = initial Rr infestation level, Pf = final Rr population density in soil.

[c] Females per 10 2.5-cm root pieces.

*, **, *** = means are significantly different at $P \leq 0.05$, 0.01, and 0.001, respectively; NS = means are not significantly different. Significance was determined after log transformation of nematode numbers from soil and eggs/root system and after square root transformation of numbers of sessile females.

Table B.30. Interrelationships between Rotylenchulus reniformis (Rr) infestation level and Diaporthe phaseolorum var. caulivora (Dpc) infection of Davis soybean with respect to number of eggs per root system.

	Eggs/root system	
Dpc	Low Rr	High Rr
present	7,181	13,533
absent	17,612	17,767
P>|t|	0.0009	0.4471

Table B.31. Effect of Diaporthe phaseolorum var. caulivora (Dpc) and Rhizoctonia solani (Rs) on egg hatch in water for Rotylenchulus reniformis (Rr) at low (500/pot) and high (1,000/pot) initial population densities.

		% Egg hatch[a]	
Treatment	Level	Low Rr	High Rr
Dpc	present	63	61
	absent	62	55
Rs	present	57	60
	absent	68	56
Source:			
Dpc		NS	NS
Rs		NS	NS
Dpc x Rs		NS	*

* = means are significantly different at $P \leq 0.05$; NS = means are not significantly different.

[a] Significance was determined after arc sin transformation of data.

Table B.32. Interaction between Diaporthe phaseolorum var. caulivora (Dpc) and Rhizoctonia solani (Rs) with respect to % egg hatch of Rotylenchulus reniformis at an infestation level of 1,000 nematodes per pot.

	% Egg hatch[a]		
	Dpc present	Dpc absent	P > |t|
Rs present	55	65	0.2179
Rs absent	67	45	0.0311
P > |t|	0.1566	0.0442	

[a] Significance was determined after arc sin transformation of data.

Interrelationships between *Rotylenchulus reniformis* and *Diaporthe phaseolorum* var. *caulivora*, and the Impact of Time of Inoculation with the Fungus

A greenhouse experiment was conducted to evaluate the interrelationships between Rr and Dpc on Davis soybean, and to determine if the time of inoculation with Dpc affected the relationship between these two pathogens. Three treatments, Dpc (present or absent), time of inoculation with Dpc (4 or 6 weeks after nematode infestation), and Rr (0, 500, or 1,000/pot), were combined in a factorial arrangement. Treatment combinations were replicated five times in a randomized complete block design.

Soil in each pot was infested with nematodes 5 days after a 10-day-old seedling was transplanted into the pot. Nematodes were allowed to develop for either 4 or 6 weeks prior to inoculation with Dpc. Dpc was introduced into the plants on infested toothpick sections inserted into a puncture made between the unifoliate and first trifoliate nodes; sterile toothpick sections were inserted into control plants. Plants were harvested 92 days after nematode infestation (62 or 48 days after inoculation with Dpc). Dpc canker lengths were measured at weekly intervals beginning approximately 14 days after inoculation. At harvest, soil and root populations of nematodes and plant weights were recorded.

Canker lengths were not influenced by either the time of inoculation with Dpc or colonization of the host by Rr (Table B.33). Expansion of cankers throughout the test period indicated that the fungus remained active.

Soil Rr populations were generally smaller on plants infected with Dpc (Table B.34). A significant Dpc x Rr interaction was detected with respect to number of juvenile Rr (Table B.34). At an infestation level of 500 Rr/pot, populations of Rr juveniles were smaller from Dpc-colonized plants than those from respective controls

Table B.33. Effect of Diaporthe phaseolorum var. caulivora (Dpc) inoculation time and Rotylenchulus reniformis (Rr) infestation level on canker length (mm) for Davis soybean inoculated with Dpc; DAI=days after inoculation with Dpc.

Treatment	Level	16/14[a] DAI[b]	20/20 DAI[b]	28/27 DAI[b]	34/34 DAI[b]	48/48 DAI
Rr	0	7.3	18.3	54.0	105.8	222.2
	500	6.6	15.2	27.6	67.6	216.6
	1,000	6.3	14.6	35.9	66.3	192.2
Time	4 wk	6.4	18.1	32.0	64.3	205.3
	6 wk	7.0	13.9	46.3	95.5	215.4
Source:						
Rr		NS	NS	NS	NS	NS
linear		NS	NS	NS	NS	NS
quadratic		NS	NS	NS	NS	NS
Time		NS	NS	NS	NS	NS
Rr x Time		NS	NS	NS	NS	NS

[a] Numbers indicate DAI for 4 wk and 6 wk inoculation times, respectively.
[b] Significance determined after natural log transformation of data.
NS = means are not significantly different ($P \leq 0.05$).

Table B.34. Effects of Rotylenchulus reniformis (Rr) infestation level, Diaporthe phaseolorum var. caulivora (Dpc), and time of inoculation with Dpc on Rr soil populations 92 days after nematode infestation on Davis soybean.

Treatment	Level	Juveniles[a]	Males	Females	Total[a]
Rr	500	94,263	8,572	759	103,594
	1,000	71,854	7,946	318	80,118
Dpc	present	70,272	6,575	733	77,580
	absent	95,844	9,944	344	106,132
Time	4 wk	71,774	7,257	422	79,453
	6 wk	94,343	9,261	655	104,259
Source:					
Rr		NS	NS	NS	NS
Dpc		*	*	NS	*
Time		NS	NS	NS	NS
Rr x Dpc		*	NS	NS	NS
Rr x Time		NS	NS	NS	NS
Dpc x Time		NS	*	NS	NS
Rr x Dpc x Time		NS	NS	NS	NS

* = means are significantly different at $P \leq 0.05$; NS = means are not significantly different.

[a] Significance determined after natural log transformation of data.

(Figure B.2). A significant Dpc x time interaction also was detected (Table B.34). Plants inoculated with Dpc 4 weeks after nematode infestation supported significantly fewer males than plants inoculated with Dpc 6 weeks after nematode infestation (Figure B.3). The time of Dpc inoculation did not appear to influence the number of juvenile or female reniform nematodes in the soil.

A significant 3-way interaction (Dpc x Rr x time) was detected with respect to the number of eggs per root system (Table B.35, Figure B.4). Four weeks after inoculation with Dpc, plants colonized by the fungus supported fewer eggs/root system at the low Rr level but more eggs/root system at the high Rr level. However, when plants were inoculated 6 weeks after nematode infestation, colonization by the fungus did not influence the number of eggs recovered from the roots at either nematode infestation level.

A significant interaction between Dpc and time was detected with regard to percent egg hatch after 5 days in sterile water (Table B.35, Figure B.5). Hatch of eggs recovered from plants inoculated with Dpc 4 weeks after nematode infestation was significantly lower than hatch of eggs collected from respective controls. However, egg hatch was not influenced by colonization with Dpc when plants were inoculated 6 weeks after nematode infestation. This suggests that a longer colonization period by Dpc is needed to reduce viability of Rr eggs.

R values (ratio of final Rr soil populations to initial Rr soil populations) were significantly larger for reniform infestation levels of 500/pot than for 1,000/pot (Table B.35). This is not surprising, since initial space and nutrient constraints are not as intense at lower population densities. Further, R values were lower on Dpc-colonized plants than on respective controls (Table B.35), indicating that the fungus is limiting nematode reproduction.

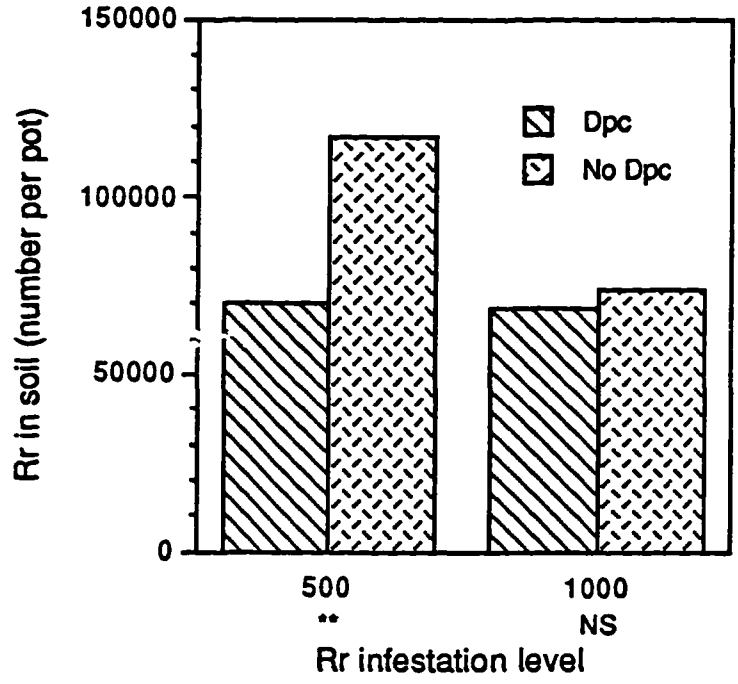

Fig. B.2. Impact of nematode infestation level and colonization by Diaporthe phaseolorum var. caulivora (Dpc) on number of Rotylenchulus reniformis juveniles recovered from the soil at harvest. ** = means are significantly different at $P \leq 0.01$; NS = means are not significantly different.

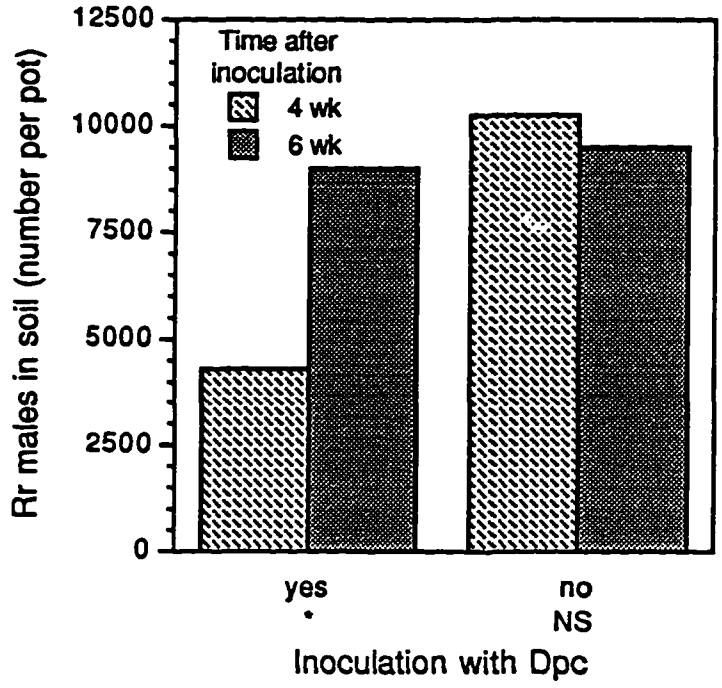

Fig. B.3. Influence of colonization by Diaporthe phaseolorum var. caulivora (Dpc) and time of inoculation with Dpc on numbers of male Rotylenchulus reniformis recovered from the soil at harvest. * = means are significantly different at $P \leq 0.05$; NS = means are not significantly different.

Table B.35. Effects of Rotylenchulus reniformis (Rr) infestation level, Diaporthe phaseolorum var. caulivora (Dpc), and time of inoculation with Dpc on Rr population parameters 92 days after nematode infestation on Davis soybean.

Treatment	Level	Eggs/root system[a]	R[a,b]	% Egg hatch
Rr	500	71,349	350.4	54.7
	1,000	52,680	132.8	53.3
Dpc	present	60,331	208.2	44.6
	absent	63,698	275.0	63.4
Time	4 wk	45,879	182.4	54.2
	6 wk	78,150	300.8	53.8
Source:				
Rr		NS	***	NS
Dpc		NS	*	*
Time		**	NS	NS
Rr x Dpc		*	NS	NS
Rr x Time		**	NS	NS
Dpc x Time		NS	NS	**
Rr x Dpc x Time		**	NS	NS

[a] Significance determined after natural log transformation of data.

[b] R = Pf/Pi.

*, **, *** = means are significantly different at $P \leq 0.05$, 0.01, and 0.001, respectively; NS = means are not significantly different.

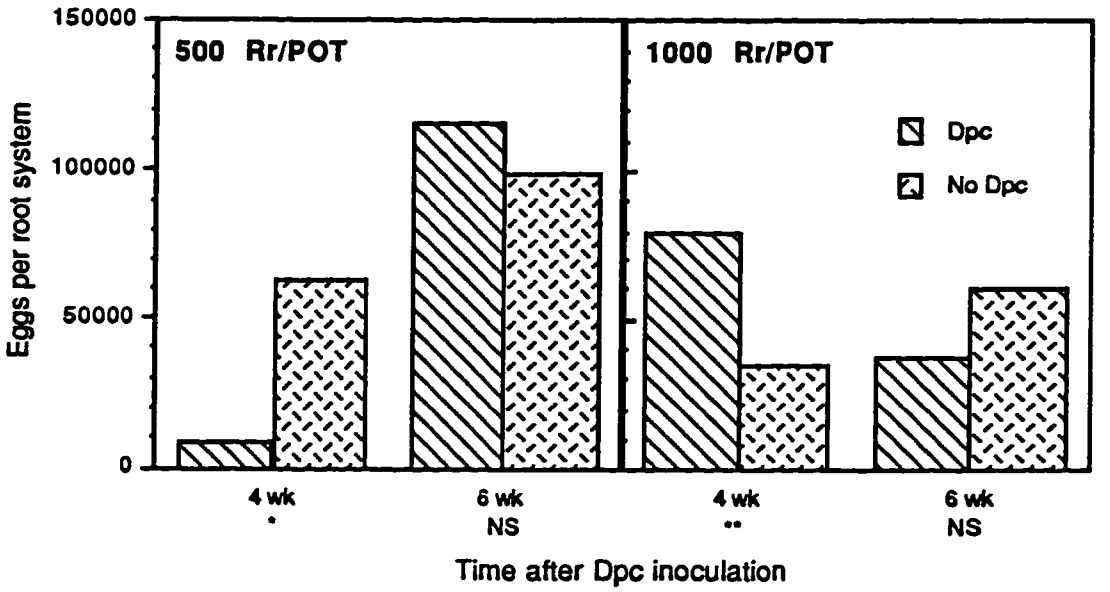

Fig. B.4. Impact of Rotylenchulus reniformis (Rr) infestation level, Diaporthe phaseolorum var. caulivora (Dpc), and time of inoculation with Dpc on the number of Rr eggs recovered from Davis soybean roots at harvest. *, ** = means are significantly different at $P \leq 0.05$ and 0.01, respectively; NS = means are not significantly different.

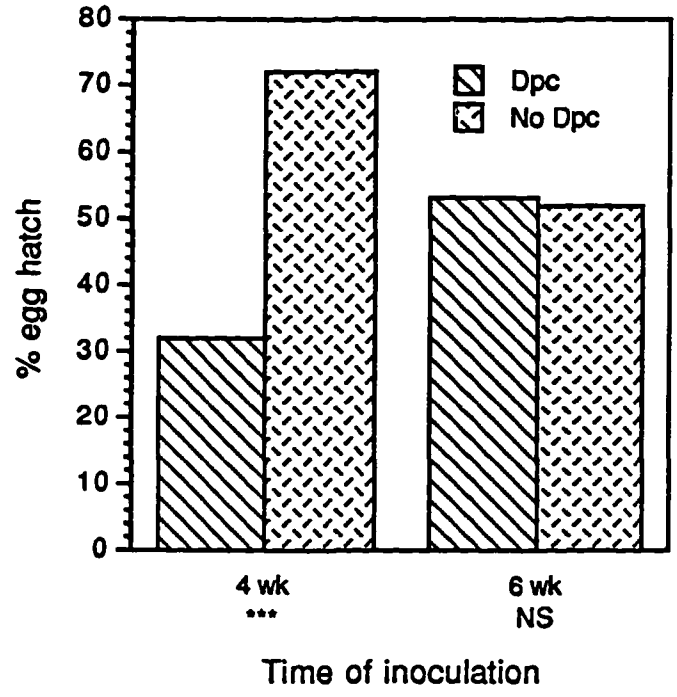

Fig. B.5. Effects of Diaporthe phaseolorum var. caulivora (Dpc) and time of inoculation with Dpc on percent hatch of Rotylenchulus reniformis eggs after 5 days in sterile water. *** = means are significantly different at $P \leq 0.001$; NS = means are not significantly different.

A significant Dpc x time interaction was detected with respect to shoot and plant dry weight (Table B.36). Shoot and plant weights were significantly lower on soybean inoculated with Dpc 4 weeks after nematode infestation than on plants inoculated 6 weeks after nematode infestation (Figure B.6). Root weight was not influenced by any of the treatments (Table B.36).

Interrelationships between *Diaporthe phaseolorum* var. *caulivora* and *Rhizoctonia solani* in Greenhouse Evaluations

A greenhouse test was conducted to examine the interrelationships between Dpc and Rs AG-1 IA on soybean. Treatments were soybean cultivar (Sharkey, Bay, Bedford, Wilstar 550), Dpc (present, absent), and Rs (present, absent) in a factorial arrangement. Each treatment combination was replicated five times in a completely randomized design. The experiment was conducted twice and data presented are combined across tests.

Dpc (Opelousas 3 isolate) was introduced into the plants on infested toothpick sections inserted into the stem between the unifoliate and first trifoliate nodes. Sterile toothpick sections inserted into a separate set of plants served as controls. Plants were inoculated with Dpc at growth stage V3. Plants were inoculated with Rs (BHIA-10 isolate) when plants were at growth stage V6 by placing a 3-mm-diam. plug of mycelium growing on APDA on each leaflet of the fifth trifoliate up from the base of the plant. Plugs of APDA without the fungus served as controls. Stem canker lesion length (canker plus wound parenchyma) and aerial blight ratings were recorded 4 weeks after inoculation with Dpc.

Rs had no influence on stem canker lesion length on Dpc-infected plants for any cultivar (Table B.37). This may be due to the short time that Rs was actually colonizing the host. Dpc did not affect the severity of aerial blight on Rs-infected plants of the cultivars Bedford and Wilstar 550. However, the severity of aerial blight was increased

Table B.36. Effects of Rotylenchulus reniformis (Rr), Diaporthe phaseolorum var. caulivora (Dpc), and time of Dpc inoculation on Davis soybean plant dry weight (g after 96 hours at 70°C).

Treatment	Level	Shoot weight	Root weight[a]	Plant weight
Rr	0	31.0	32.3	63.4
	500	34.3	46.1	80.4
	1,000	32.6	41.1	73.8
Dpc	present	27.6	41.2	68.8
	absent	37.7	38.5	76.3
Time	4 wk	31.3	35.5	66.8
	6 wk	34.0	44.2	76.3
Source:				
Rr		NS	NS	NS
Dpc		***	NS	NS
Time		NS	NS	NS
Rr x Dpc		NS	NS	NS
Rr x Time		NS	NS	NS
Dpc x Time		**	NS	*
Rr x Dpc x Time		NS	NS	NS

[a] Significance determined after square root transformation of data.

*, **, *** = means are significantly different at $P \leq 0.05$, 0.01, and 0.001, respectively; NS = means are not significantly different.

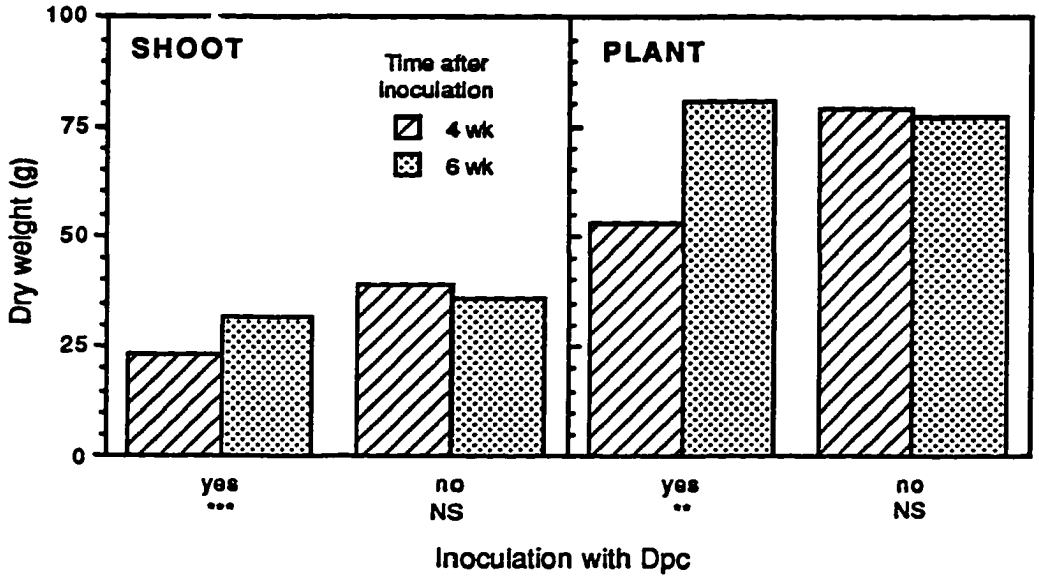

Fig. B.6. Effect of colonization by Diaporthe phaseolorum var. caulivora (Dpc) and the time of inoculation with Dpc on Davis soybean shoot and plant dry weights. **, *** = means are significantly different at $P \leq 0.01$, and 0.001, respectively; NS = means are not significantly different.

Table B.37. Influence of Rhizoctonia solani (Rs) on stem canker lesion length (mm) for four soybean cultivars infected with Diaporthe phaseolorum var. caulivora.

	Cultivar			
Rs	Wilstar 550	Bedford	Bay	Sharkey
absent	8	35	26	28
present	9	36	37	14
$P > \|t\|$	NS	NS	NS	NS

Data from two tests combined.
NS = means are not significantly different ($P \leq 0.05$).

by Dpc on the cultivars Bay and Sharkey (Table B.38). The specific factors to which this increase can be attributed were not determined.

Table B.38. Influence of inoculation with Diaporthe phaseolorum var. caulivora (Dpc) on aerial blight severity (% of leaf affected) for four soybean cultivars 96 hours after inoculation with Rhizoctonia solani.

	Cultivar			
Dpc	Wilstar 550	Bedford	Bay	Sharkey
absent	14	9	9	5
present	9	9	22	13
$P > \|t\|$	NS	NS	*	*

Data from two experiments combined.

* = means are significantly different at $P \leq 0.05$; NS = means are not significantly different.

VITA

Salliana Ryan Stetina was born on February 7, 1966 in Denver, Colorado. She received her secondary education at Cary Grove High School in Cary, Illinois, graduating in the spring of 1984. In the fall of 1984 she entered Eastern Illinois University in Charleston, Illinois, majoring in Zoology and minoring in Botany. She received her B.S. degree in the spring of 1988, graduating Magna Cum Laude and with University Honors. In the fall of 1988, she was awarded a Teaching Assistantship in the Botany Department at Eastern Illinois University and pursued a M.S. degree, graduating in the summer of 1990. In the fall of 1990, she entered Louisiana State University on a College of Agriculture Graduate Assistantship. In the fall of 1991, she began a doctoral program in Plant Pathology in the Department of Plant Pathology and Crop Physiology under the direction of Drs. J. P. Snow, E. C. McGawley, and J. S. Russin. In the spring of 1993, she joined the staff of the Department of Plant Pathology and Crop Physiology as a Research Associate/Specialist with the rice pathology program. She married Kenneth Charles Stetina in June, 1996. She has served as the President of the Plant Pathology and Crop Physiology Graduate Student Association and has been the student representative on the departmental Courses and Curricula and Ad-Hoc/Teaching Lab Improvement committees. She is a member of Beta Beta Beta Biological Society, Phi Sigma Society for the Biological Sciences, and the Society of Nematologists. She is now a candidate for the Doctor of Philosophy degree in Plant Health (Plant Pathology).

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Salliana Ryan Stetina

Major Field: Plant Health

Title of Dissertation: Interrelationships between Meloidogyne Incognita and Rotylenchulus Reniformis on Soybean

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Gerard T. Berggren Jr.

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Date of Examination:

September 13, 1996